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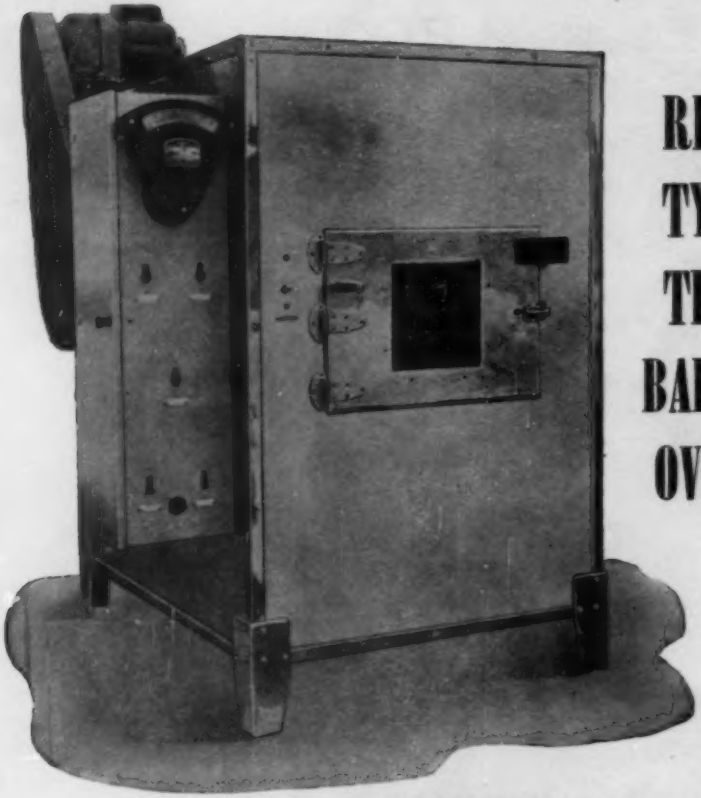

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CEREAL CHEMISTRY

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DETERMINATION OF YEAST GROWTH IN DOUGHS[1]

J. A. THORN[2] AND J. W. ROSS[3]

ABSTRACT

Yeast cells were quantitatively recovered from dough by a process in which the dough was blended, the starch was gelatinized by heat-treatment, and the major part of the starch and protein was solubilized by digestion with fungal amylase and protease. After removal of lipids by an acid extraction with ethanol-ether, a further enzymatic digestion provided a suspension containing the yeast cells but very little extraneous material. The cells were readily counted in a Petroff-Hausser chamber, and buds were easily distinguished. The method was used to determine the amount of growth undergone by compressed and active dry yeasts in various types of commercial doughs. In sponges, both yeasts grew about 50–60% in 4 hours, but no further increase occurred in the sponge doughs. The yeasts grew about 35% in straight doughs fermented 3–3.5 hours. Most of the bud formation occurred during pan proof. No growth was found in sweet dough sponges or in flour brews fermented 3.5–4 hours. Because recovery varied during the course of dough fermentation, the nitrogen content of the isolated yeast could not be used as an indication of growth.

The growth of yeast in doughs has been investigated thoroughly only in the case of straight dough fermentations. In 1941, Hoffman, Schweitzer, and Dalby (3) described a method for counting yeast cells in dough in which the cells were washed out of the dough with salt solution and were subsequently counted in a hemocytometer. In an accompanying publication (4), the effects on yeast growth of fermentation time, yeast concentration, and various inorganic salts were described. In calculating the amount of growth, yeast buds were counted as single cells. In view of the large size which buds attain in fermenting doughs, this practice would appear well founded since the buds must contribute significantly to the gassing activity of the yeast.

In their studies, Hoffman *et al.* found that compressed yeast multi-

[1] Manuscript received October 21, 1959. Contribution from Red Star Yeast and Products Co., Milwaukee, Wis.

[2] Director, Cereal Chemistry Laboratory, Research Department.

[3] Present address: Squibb Institute for Medical Research, New Brunswick, N. J.

plied from 29 to 88% in 6 hours when used at levels ranging from 2.0 to 0.5%. These results compared well with those of Simpson who, in 1936, observed yeast growth of 9–131% in straight doughs containing from 2.0 to 0.25% compressed yeast (6). More recently, Carlin (1) reported that no multiplication occurred in either straight or sponge doughs when commercial levels of yeast were employed. The method of analysis was not mentioned, but probably involved a plating technique which of course would not measure bud formation.

Because of the prevalent use of sponge doughs for the commercial production of various baked goods, and the increasing use of other fermentation systems such as brew doughs, it was of interest to us to study the growth of yeast in some of these systems. Also, no comparative data on the growth of compressed and active dry yeasts have appeared in the literature. In this paper, a method for isolating yeast cells from dough by enzymatic degradation of the insoluble starch and protein will be described, together with the results of its application to sponge, straight, sweet, and brew doughs made with compressed and active dry yeasts.

Materials and Methods

Degradation of Dough Samples. The method used in isolating yeast cells from dough samples was analogous to that of Simpson (5) in that it employed enzymatic degradation of the insoluble dough constituents (other than yeast). The suspensions of cells so obtained were practically devoid of extraneous material, and were well suited for cell count determinations with a hemocytometer.

A suitable sample of dough (20–40 g., depending upon the yeast concentration) was macerated with 80 ml. of water for 30 seconds in a Waring Blendor. The sides of the jar were scraped down with a rubber policeman and a minimum amount of water, and the suspension was blended for 60 seconds additionally. It was then transferred to a 250-ml. graduated cylinder and made up to volume. After thorough mixing, duplicate 25-ml. aliquots were placed in 50-ml. centrifuge tubes.

The aliquots were then heated in a boiling-water bath for 2.5 minutes with constant stirring. This killed the yeast cells and gelatinized the starch. The suspensions were then cooled, and to each were added 5 ml. of a fungal protease solution. This was prepared by extracting 3.5 g. of Rhozyme A-4[4] with 30 ml. of water for 30 minutes at room temperature, then removing insoluble materials by centrifuging

[4] Rohm and Haas Co., Philadelphia, Pa.

for 10 minutes (all centrifugations in this work were made with an International Equipment Co. clinical centrifuge running at top speed). The enzyme-dough mixtures were then adjusted to pH 4.8 with 0.3*N* hydrochloric acid, and were incubated in a 50°C. water bath for 60 minutes with occasional stirring.

This preliminary digestion solubilized the major portion of the protein and starch of the dough, but sufficient remained to interfere with the counting of the yeast cells. The residual material was resistant to further enzymatic hydrolysis, but was readily hydrolyzed after lipids were removed by an acidic ethanol-ether extraction.

Therefore, at the end of the first digestion period, the samples were cooled and each was transferred to a 250-ml. Erlenmeyer flask with the aid of 30 ml. of ethanol. To each flask were added 2 ml. of concentrated hydrochloric acid and 50 ml. of ether. The mixtures were shaken vigorously and, when phase separation occurred, the ether layers were siphoned off and discarded. Another 30 ml. of ethanol and 50 ml. of ether were added to each flask with shaking, followed by 22 ml. of water and further shaking. The upper phases were again discarded and the aqueous suspensions were centrifuged. The supernates were discarded, and the residues washed twice with centrifugation.

The washed residues were then suspended in about 25 ml. of water, 7.5 ml. of protease solution were added to each, and after being adjusted to pH 4.8 with hydrochloric acid, they were incubated at 50°C. for 90 minutes. The digested suspensions were centrifuged, and the solids washed twice with centrifugation; they were then made up to 50 ml.

Cell Counts. Twenty-five milliliters of the final suspension were

TABLE I

FERMENTATION SYSTEMS

	Sponge Process		Sweet Dough Sponge	Straight Dough	Brew Process	
	Sponge	Dough			Brew	Dough
	%	%	%	%	%	%
Flour	66.7	33.3	65	100	8.3	91.7
Water	42	25	43	70	33.6	32.1
Yeast food[a]	0.5	..	0.5	..	0.5	0.17
Yeast, active dry	0.9	..	2.4	0.675	1.2	..
Or yeast, compressed	2.25	..	6.0	2.0	3.0	..
Sugar	..	4.0	..	4.0	1.0	5.0
Salt	..	2.0	..	2.0	1.0	1.0
Nonfat dry milk	..	4.0	..	4.0	..	4.0
Shortening	..	3.0	..	3.0	..	3.0
Calcium carbonate	..	..	..	..	0.04	..

[a] A product of Red Star Yeast and Products Co. containing 0.3% potassium bromate and 9.7% ammonium chloride.

treated with 5 ml. of methylene blue solution (200 mg. methylene blue and 54 g. of potassium dihydrogen phosphate dissolved in 200 ml. of water). Cell counts were obtained with a Petroff-Hausser counting chamber and a microscope with 600× magnification. At least 10 fields of 90 smallest squares were counted for each sample, each field normally containing 50 to 150 yeast cells. Every bud large enough to be recognizable was also counted.

Dough Formulas. Yeast growth was determined in the fermentation systems shown in Table I.

The sponges were set at 26°–27°C. and fermented 4 hours. The sponge doughs received about 40 minutes of combined floor time and intermediate proof, followed by 50 to 60 minutes of pan proof at 35.6°C.

Sweet dough sponges were fermented 3.5 hours. Growth of yeast in the doughs was not determined.

The straight doughs were allowed three rises, followed by pan proof of 50–60 minutes. Total fermentation time was 3.0–3.5 hours.

Brews were fermented 3.5 hours at 30°C., and the doughs received about 50 minutes of combined floor time and intermediate proof and 55–65 minutes of pan proof.

Results and Discussion

The recovery of yeast cells from doughs was quite satisfactory, as shown in Table II. The data are for recoveries of compressed yeast from straight doughs and sponges sampled immediately after mixing.

TABLE II

RECOVERY OF YEAST CELLS FROM DOUGHS

DOUGH	YEAST ADDED	YEAST FOUND	RECOVERY
	millions/g	*millions/g*	%
Straight dough	239	243	102
	239	231	97
	239	231	97
Sponge	450	452	100
	450	428	95

Direct counts of yeast samples showed that compressed yeast contained about 22.1 × 10^9 cells per g., and active dry yeast (ADY) 38.7 × 10^9 per g. (both on an "as-is" basis). The ADY cells are larger and somewhat more active than compressed yeast cells, and to provide the same fermentation rate fewer ADY cells are needed than CY cells.

Some other factors affecting fermentation rate, including the relative amounts of the two types of yeast needed for equal rates, have been discussed elsewhere (7).

In this paper, growth of yeast is defined as cell increase, as evidenced either by an increase in the number of single cells or by the formation of buds. In reality, very little division occurred and nearly all growth consisted of bud formation. Toward the end of most of the dough fermentations, the majority of the buds were very large and often equal in size to the mother cells. It seemed reasonable, therefore, that the buds played a significant role in the over-all gassing activity of the yeast.

Table III shows the growth of yeast in straight doughs. Three different lots of compressed yeast were compared with three lots of ADY. It is apparent that the two types of yeast grew to the same extent.

TABLE III
GROWTH OF YEAST IN STRAIGHT DOUGHS

Yeast	Cells (Millions per g. of Dough)						Over-All Increase
	Zero-Time		End of Third Rise		End of Proof		
	Cells	Buds	Cells	Buds	Cells	Buds	
							%
CY-A	239	4	225	16	229	85	
CY-B	226	5	222	35	241	92	
CY-C	224	7	226	31	225	92	
Av.	230	5	224	27	232	90	37
ADY-A	163	1	147	13	167	56	
ADY-B	145	3	134	22	133	51	
ADY-C	143	4	139	32	137	63	
Av.	150	3	140	22	146	57	33

The proportion of buds to mother cells at the end of proof was the same for both yeasts (39%). The data also show that most of the budding occurred during the proof stage, i.e., in the last hour of fermentation.

Growth characteristics of compressed yeast and ADY in sponge doughs are illustrated in Table IV. All or nearly all of the growth occurred during the sponge fermentation, the cell counts changing but little during the dough fermentation. As in the case of straight doughs, little difference was noted between compressed yeast and ADY. In general, it was found that both types of yeast form 50 to 60% buds in the 4-hour sponge fermentation. Most of the buds formed

in the first 3 hours. For example, in one set of experiments in which both yeasts grew 59% in 4 hours, the proportion of buds at 3 hours was 50% in the case of compressed yeast, and 51% in the case of ADY. The almost complete cessation of cell growth when the sponges were converted to doughs may have represented a lag phase engendered by the rather drastic change in the yeasts' environment.

TABLE IV

GROWTH OF YEAST IN SPONGE DOUGHS

YEAST	CELLS (Millions per g. of Dough)								OVER-ALL INCREASE
	SPONGE				DOUGH				
	Zero-Time		4 Hours		Zero-Time		End Proof		
	Cells	Buds	Cells	Buds	Cells	Buds	Cells	Buds	
									%
ADY-A	344	2	316	124	212	82	213	74	
ADY-B	313	6	330	114	215	79	233	93	
Av.	329	4	323	119	214	81	223	84	50[b]
			expected[a]		197	73			
CY-A	446	6	474	236	296	148	316	127	
CY-B	421	7	437	223	250	130	274	117	
Av.	434	7	456	230	273	139	295	122	55[b]
			expected[a]		277	140			

[a] Calculated from counts found for 4-hour sponges.
[b] Based on total population (cells + buds) in dough at end of proof compared to that initially in sponge.

TABLE V

YEAST POPULATIONS IN SWEET DOUGH SPONGES

YEAST	CELLS (Millions per g.)			
	ZERO-HOUR		3.5 HOURS	
	Cells	Buds	Cells	Buds
ADY	800	27	790	21
CY	1090	33	1060	47

Neither compressed yeast nor ADY grew in the sweet-dough sponges, as shown in Table V. These sponges were fermented 3.5 hours, and contained typically high levels of yeast, the yeast:flour ratio being about 2.7 times as great as that in regular or pan bread sponges. The reason for lack of growth is not known, but the results agreed qualitatively at least with those of Simpson (6) and Hoffman *et al.* (4), who found growth to decrease as yeast concentration was raised.

Examination of flour brews at the end of the 3.5-hour fermentation period showed that growth was obtained with neither compressed yeast nor ADY in this system. With compressed yeast, for example,

TABLE VI
GROWTH OF YEAST IN BREW DOUGHS

YEAST	CELLS (Millions per g.)				OVER-ALL INCREASE
	ZERO-TIME		END PROOF		
	Cells	Buds	Cells	Buds	
ADY	284	6	293	55	20%
CY	386	5	388	63	16%

the proportion of buds at the end of the fermentation was 3% as compared to 4% at the start. There was no change in the number of single cells. These results agreed with those of Choi (2), who reported that compressed yeast did not bud or multiply in milk-buffered brews. Growth did occur in the doughs made with flour brews, however, as Table VI shows. While the amount of budding was relatively small, its occurrence was unexpected in view of the lack of growth found in sponge doughs.

When these studies were initiated, it was hoped that changes in the nitrogen content of the isolated cells could be used as a second index of growth. Analyses of dough samples taken immediately after mixing showed that the degradation procedure eliminated about 99.5% of the flour nitrogen but only 30% of the yeast nitrogen. Further experiments, however, showed that the recovery of yeast nitrogen varied considerably during straight dough fermentations, presumably because of the changes in the types and distribution of nitrogenous compounds in the actively growing yeast. Because of this inconstancy, the nitrogen values could not be used as a criterion of cell growth.

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VITAL WHEAT GLUTEN BY DRUM DRYING
II. Pilot-Plant Studies and Cost Estimates[1]

CHARLES VOJNOVICH, V. F. PFEIFER, R. A. ANDERSON, AND E. L. GRIFFIN, JR.

ABSTRACT

Conditions and equipment suitable for the production of dry vital wheat gluten by drum-drying wet gluten dispersed in dilute acetic acid were studied. In these tests a satisfactory product was obtained by completely dispersing wet gluten in dilute acetic acid at a pH from 4.3 to 5.1 and a solids concentration of 12–20% by weight, by drum-drying the dispersed gluten at atmospheric pressure and a temperature about 260°F. (127°C.), and by grinding the dried material in an attrition or hammer mill. The process uses standard equipment, and it can be readily adapted in industrial plants to make dry vital gluten for baking or other food purposes, or for the preparation of industrial chemicals. Cost estimates for drying 6 million lb. of vital gluten product annually by this process show a total drying cost of 4 cents per lb. of dry material in a plant costing about $477,000.

Descriptions of processes for producing vital dry gluten by drum-drying are limited. Miley *et al.* (3) patented a process in which wet gluten is dispersed in a mixture of water and carbon dioxide under pressure and the resulting dispersion is dried in a drum-dryer, spray-dryer, etc. Tuomy and Slotter (5) described a method in which wet gluten is dispersed in ethanol and dried on a drum-dryer. An earlier article (4) discussed preliminary investigations of drum-drying wet gluten dispersed in dilute acid. This paper describes pilot-plant investigations employing various types of equipment and optimum process conditions. Cost estimates are presented for drum-drying vital gluten on a commercial scale.

Equipment

Laboratory dispersions of gluten in dilute acetic acid were prepared in a 1-gal. Waring Blendor[2] (Waring Products Co., New York, N. Y.) Model CB-3 operated at 20,000 r.p.m. Pilot-plant dispersions were prepared with the following: a stainless-steel ribbon blender having a capacity of about 19 gal. and a rotational speed of 70 r.p.m.; a pump recirculation system comprising a 1-¼-in. Bump Pump (Ulrich Mfg. Co., Roanoke, Illinois) Model 15 (single-lobe offset impellers

[1] Manuscript received October 1, 1959. Contribution from the Northern Regional Research Laboratory, Peoria, Illinois. This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

[2] Mention of trade names or equipment does not constitute endorsement by the U. S. Department of Agriculture over others of a similar nature not mentioned.

operating at 160 r.p.m.) and a 40-gal. stainless-steel tank; a Cowles Dissolver (Morehouse-Cowles, Inc., Los Angeles, Cal.) Model 7VT equipped with a 10-in. stainless-steel impeller rotating at 1,800 r.p.m.; a Morden Stock Maker (Morden Machines Co., Portland, Ore.) Model MLB-4 equipped with a vaned conical rotor and a stator with variable clearance, having a maximum capacity of 1.5 gal., and operated at 1,400 r.p.m.; a Premier Colloid Mill (Premier Mill Co., Geneva, N. Y.) Model UB-5, equipped with a 3-in. tapered disk rotating at 17,000 r.p.m. in an adjustable stator; and a Manton-Gaulin Homogenizer (Manton Gaulin Mfg. Co., Everett, Mass.) Model 25 M-3BA, of the orifice type, operating with homogenizing pressure up to 5,000 p.s.i.g.

Drum-drying of gluten dispersions was carried out on Buflovak laboratory and pilot-plant double drum-dryers (Buffalo Machine and Foundry Co., Buffalo, N. Y.) at atmospheric pressure. The rolls of both models were constructed of chrome-plated cast iron. The laboratory drum-dryer had two rolls each 6 in. in diameter by 7⅝ in. long with a total area of 2.0 sq. ft. Rolls in the pilot-plant dryer were 2 ft. in diameter by 3 ft. long and had a total area of 36 sq. ft. Steam pressure was adjustable from 2 to 120 p.s.i.g. for both dryers; clearance between the drums could be varied for each, and rotational speed was variable from 2 to 18 r.p.m. for the laboratory dryer, and from 1 to 8 r.p.m. for the pilot-plant dryer.

Drum-dried gluten was ground in a Mikro-Samplmill (Pulverizing Machinery Co., Summit, N. J.) equipped with screens having 0.020-, 0.042-, and 0.0625-in. holes, in a Raymond hammer mill (Combustion Engineering, Inc., Chicago, Ill.) equipped with a screen having 0.033-in. holes, and in a Labconco Heavy-Duty Attrition Mill (Laboratory Construction Co., Kansas City, Mo.) using a fine clearance.

Experimental Methods

To ensure that a wide range of physical characteristics was covered, patent, straight, first-clear, and second-clear flours were used to prepare wet gluten by the "batter process" as described by Anderson, Pfeifer, and Lancaster (2). These flours ranged in ash content from 0.41 to 1.95% on a dry basis. Wet glutens were produced containing 67 to 70% moisture and 60 to 85% total protein on a dry basis for these drying tests.

Prior to pilot-plant drying, samples of wet gluten were tested from each run to determine their dispersibility. Dispersibility tests were made by mixing wet gluten, distilled water, and glacial acetic acid in the Waring Blendor for about 4 minutes at several pH levels. The

dispersed gluten samples were drum-dried, and the dry products tested for rehydration properties. The optimum pH level as shown by this test was used in the pilot-plant for that particular gluten. A sample of the wet gluten from each batch was also dried in a vacuum oven at 100°F. (38°C.) at 1 cm. Hg absolute for 16 hours for use as a vital gluten standard to determine the comparative quality of drum-dried products.

Pilot-plant dispersions were prepared by a variety of batch and continuous methods, using between 1.5 and 7.5% glacial acetic acid, based on the weight of dry gluten.

In the batch methods wet gluten was mixed with equal weights of ion-exchange water (hydrogen-cycle) containing the proper amount of acetic acid, and the dispersions were made as follows: in the ribbon blender, 3½ hours; Bump recirculating system, 2½ to 3 hours; Cowles dissolver, 30 to 90 minutes; Morden Stock Maker, 15 to 17 minutes; Premier colloid mill at 0.010-in. clearance, recirculation of a premixed slurry from the Cowles dissolver; Manton-Gaulin mill at 3,500 p.s.i.g. homogenizing pressure, recirculation of a premixed slurry from the Cowles dissolver.

In the continuous methods, wet gluten and dilute acetic acid were premixed batchwise in the Cowles dissolver for 7 to 9 minutes until the mixture became a uniform and fluid slurry. This slurry, containing about 16.5% solids, was fed to either the Morden or the Manton-Gaulin mill. When the Morden mill was used, the slurry was fed into the bottom of the Stock Maker until it was full. After 10 minutes, dispersion feed was pumped into the mill at such a rate that the dispersed gluten was displaced in about 10 minutes. The gluten dispersion was removed continuously from the top of the mill. When the Manton-Gaulin homogenizer was used, the slurry was recirculated and homogenized for 5 minutes, after which feed was pumped into the bottom of the mill container at a continuous rate so as to displace the homogenized gluten in 5 minutes. The gluten dispersion was removed from a constant level near the top of the homogenizer container.

Dispersed gluten was dried at a steam pressure of 20 p.s.i.g., either on the laboratory dryer at a rotational speed of about 12 r.p.m. or at 6 r.p.m. on the pilot-plant dryer, to produce 0.8 lb. or more of dry material per sq. ft. of drying area per hour. The roll clearance was set wide enough to give a dry gluten containing about 6% moisture. The vital product was scraped from the rolls and ground in an attrition or hammer mill at a temperature below 122°F. (50°C.). (See Fig. 1.)

The quality of the standard vacuum-dried and drum-dried glutens

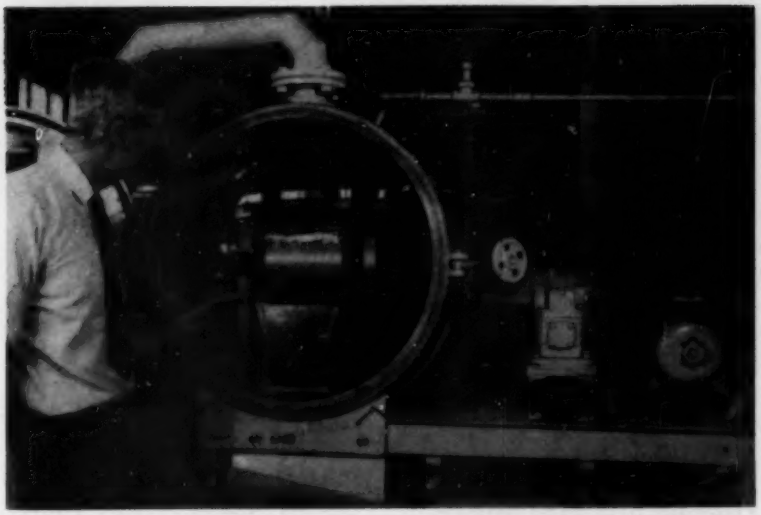

Fig. 1. Gluten dispersed in dilute acetic acid is dried on a laboratory drum-dryer at atmospheric pressure to yield a vital product.

was estimated from their nitrogen solubility in 0.1*N* acetic acid, the stretch and feel of gluten when rehydrated with water or buffer (4), and the results of baking tests. The vacuum-dried control usually has a nitrogen solubility of 95 to 98% and is arbitrarily assigned a rehydration value of 4. Rehydration is expressed on an arbitrary scale of 0, 1, 2, 3, or 4. Zero describes material so completely denatured that the gluten will not agglomerate, and 4 describes materials that behave like the vacuum-dried product. Baking quality of the glutens was determined by mixing dried gluten with a 13.5% protein spring wheat flour to obtain a blend of approximately 19.1% protein content. This proportion was equivalent to 10% of dried gluten (containing 75% protein, dry basis) and 90% of flour. The improved Kjeldahl method for nitrate-free samples (1) was used to determine protein (N × 5.7). Moisture was determined by heating a 2-g. sample in a vacuum oven at 100°–110°C. for 4 hours.

Results and Discussion

Effect of Type of Water Used in Dispersion. Hard, distilled, or deionized water, as well as water treated by ion-exchange (hydrogen-cycle) or by zeolite (sodium-cycle) can be used with glacial acetic acid in dispersing gluten for drum-drying (Table I). These dispersions were made in a Waring Blendor and were drum-dried with a roll

TABLE I

EFFECT OF WATER USED TO DISPERSE GLUTEN[a] PRIOR TO DRUM-DRYING

TYPE OF WATER USED FOR DISPERSION IN WARING BLENDOR	pH OF WATER	DISPERSION[b]		DRUM-DRIED GLUTEN[c]	
		HAc Used, Percent of Dry Gluten	pH	Rehydration Test	Nitrogen Soluble in 0.1*N* HAc
		%		*arb. units*	%
Distilled	6.4	3.8	4.4	3[d]	84[d]
Deionized	6.1	3.8	4.4	3	86
Ion-exchange, hydrogen cycle	3.3	3.8	4.4	3	88
Zeolite-treated (sodium cycle)	7.1	5.0	4.3	3	87
Tapwater (500 p.p.m. total hardness)	7.2	5.0	4.3	3	86

[a] Gluten from second-clear flour; ash content 1.32%, protein content 73% (d.b.).
[b] Dispersions contained 16.5% by wt. solids.
[c] Dried gluten contained 72.7% total protein (d.b.).
[d] Soluble nitrogen 96% and rehydration value 4 for vacuum-dried gluten.

temperature of 267°F. (130°C.). However, zeolite-treated (sodium-cycle) and hard water required more acid than the other types. For preparing dispersions hydrogen-cycle ion-exchange water is recommended.

Effect of Dispersing Equipment. Either the Morden Stock Maker or the Manton-Gaulin homogenizer used in the batch or continuous methods is suitable for dispersing gluten in dilute acid as shown in Table II. Their vigorous action minimizes dispersing time and gluten degradation. The Cowles dissolver is suitable for preparing a gluten premix for further processing in a disperser. Batch methods for dispersing gluten using the ribbon blender, the pump recirculating system, or the Cowles dissolver required a long time to disperse the gluten and the product lost elasticity because of degradation. Long dispersion times usually resulted in drum-dried products of reduced rehydration values, although nitrogen solubilities were usually satisfactory.

In most cases dispersions made with high-protein gluten, 80% or higher dry basis, gave the best drum-dried products. Dispersions containing up to 20% solids can be made with high-protein gluten in the Morden Stock Maker, and drum-dried products of good quality are obtained.

Drum-Dryer Operating Conditions. Laboratory results indicate that highest drying rates were obtained at low steam pressure, wide roll clearance, high rotational speed, and with a feed puddle maintained between the rolls (Table III). More gluten was dried per sq. ft. of area at a lower temperature than at a higher one because a thicker gluten film adhered to the rolls. A drying temperature of

TABLE II

Effect of Equipment Used for Dispersing Wet Gluten Prior to Drum-Drying

Flour		Dispersion			Drum-Dried Gluten[a]		
Grade	Ash	Solids Content	Dispersing Time	pH	Total Protein	Rehydration Test	Nitrogen Soluble in 0.1*N* HAc
	% by wt. (d b)	*% by wt.*	*minutes*		*% (d b)*	*arb. units*	*%*
				Morden Stock Maker			
Straight	0.41	16.5	15	4.8	79.5	3½ (3)[b]	91 (93)[b]
Second clear	1.32	16.5	17	4.5	72.7	2½ (3½)	85 (85)
Second clear	1.32	20.0	17	4.5	72.7	2½ (3½)	82 (85)
Second clear	1.32	16.5	17	4.6	80.8	3½ (3½)	95 (94)
Second clear	1.32	20.0	17	4.4	80.8	3 (3½)	94 (94)
Second clear	1.50	16.5	12[c]	4.4	81.3	3½ (3½)	91 (93)
				Manton-Gaulin			
Second clear	1.50	16.5	5[d]	4.6	78.2	3 (3½)	87 (87)
Second clear	1.50	16.5	21[e]	4.6	76.3	3½ (3½)	89 (85)
				Ribbon blender			
First clear	0.79	16.5	210	4.8	84.1	2½ (4)	78 (95)
				Pump recirculation			
First clear	0.79	16.5	150	4.8	81.8	3 (3½)	84 (89)
Second clear	1.47	16.5	180	4.5	70.1	1½ (2)	85 (92)
Second clear	1.47	16.5	20	4.5[e]	70.1	1½ (2)	83 (92)
Second clear	1.47	16.5	20	4.5[f]	70.1	1½ (2)	83 (92)
				Cowles dissolver			
Patent	0.41	16.5	30	4.5	79.9	3 (3½)	84 (92)
Patent	0.41	16.5	60	4.5	79.9	3 (3½)	88 (92)
Patent	0.41	16.5	90	4.5	79.9	3½ (3½)	92 (92)
Second clear	1.36	16.5	30	4.7	71.6	3 (3)	87 (93)
Second clear	1.36	16.5	60	4.7	71.6	2½ (3)	93 (93)
Second clear	1.36	16.5	90	4.7	71.6	2 (3)	93 (93)

[a] Soluble nitrogen 96% and rehydration value 4 for vacuum-dried glutens.
[b] Parenthesized figures are values obtained with dispersions prepared in Waring Blendor.
[c] Continuous. Gluten and acid premixed with Cowles dissolver for 8 minutes.
[d] Batchwise. Gluten and acid premixed with Cowles dissolver for 8 minutes.
[e] Dispersion passed once through Premier colloid mill.
[f] Dispersion passed once through Manton-Gaulin homogenizer.

TABLE III

Drum-Drying Rates for Gluten Dispersions[a]

Flour		pH of Gluten Dispersion	Drum-Dryer Operation[b]			Drum-Dried Gluten			
Grade	Ash		Roll Clearance	Roll Speed	Rate[c]	Moisture Content	Total Protein	Rehydration Test	Nitrogen Soluble in 0.1*N* HAc
	% by wt. (d b)		*inches*	*rpm*	*lb/hr/ft²*	*%*	*% (d b)*	*arb. units*	*%*
Patent	0.40	4.6	0.028	12	0.90	8.2	79.9	3½[d]	92[d]
	0.41	4.6	0.025	12	0.91	9.0	74.9	3	91
First clear	0.79	4.7	0.018	17	1.27	4.4	81.4	3½	88
	0.79	4.6	0.030	12	0.82	6.7	78.5	4	95
	0.79	4.7	0.028	12	0.90	6.1	83.2	3½	93
Second clear	1.32	4.4	0.047	12	0.84	6.0	80.8	3½	94
	1.32	4.5	0.051	12	0.99	6.0	80.8	3½	92
Second clear	1.32	4.4	0.032	18	1.18	6.2	75.0	3	86
	1.32	4.4	0.042	12	0.94	6.3	75.0	3	86
	1.32	4.5	0.037	12	0.52[e]	6.5	75.0	2½	82

[a] Dispersions contained 16.5% by wt. solids.
[b] Drying temperature 260°F. (127°C.).
[c] Pounds of dry material produced per hour per sq. ft. of roll surface.
[d] Soluble nitrogen 96% and rehydration value 4 for vacuum-dried glutens.
[e] No puddle maintained between the rolls.

about 260°F. (127°C.) was found to be most suitable for drum-drying. To obtain the best drying rate, the roll clearance was set just short of the spacing that gave a slightly damp drum-dried product. The roll clearance required thus roughly indicated the quality of the dried gluten. When a wide clearance could be used, the quality of the product was usually good; when a narrower clearance was required, the quality was usually poor, but the drying rate was increased.

Large batches of dispersed gluten dried on the pilot-plant drum-dryer resulted in products similar to those obtained on the laboratory dryer. However, the rotational speed of 6 r.p.m. on the larger dryer gave a slightly lower drying rate. Increasing the rotational speed of the rolls to 10 or 12 r.p.m. is necessary to raise the drying rate of the pilot-plant dryer to 1 lb. or more of dry material per hour per sq. ft. of drying area.

Grinding of Dry Gluten. Vacuum-dried gluten ground in either the hammer mill or the laboratory Mikro-Samplmill resembles available commercial vital glutens both in bulk density and in particle size (Table IV). When ground in a laboratory attrition mill, the vacuum-

TABLE IV
GRINDING DRIED GLUTEN IN VARIOUS MILLS

Type	Grinding		Ground Gluten	
	Mill	Screen Openings	Sieve Analysis: Through 100-mesh	Bulk Density
		inches	%	*g/ml*
Vacuum-dried	Attrition	....	4	0.76
	Hammer	0.033	62	.76
	Mikro-Samplmill	0.020	51	.78
Drum-dried	Attrition	....	63	.42
	Hammer	0.033	92	.57
	Mikro-Samplmill	0.020	94	.54
Commercial No. 1	.....	....	61	.79
No. 2	....	....	68	.80
No. 3	.....	....	71	0.82

dried gluten is too coarse to pass a 100-mesh screen. Drum-dried gluten yields finer products in any of the three mills. Bulk densities vary from 0.42 to 0.57 g. per ml. for drum-dried glutens as compared to 0.79 to 0.82 g. per ml. for commercial vital glutens. To grind drum-dried gluten in the hammer mill, 33 watt-hours were consumed per lb. of dry product, whereas in the laboratory attrition mill only 27 watt-hours were required. When vacuum-dried gluten was ground in the hammer mill, 39 watt-hours were consumed per lb. of dry product.

It is expected that in commercial mills drum-dried gluten would take considerably less power for grinding than would vacuum-dried.

Drum-dried gluten overheated when ground in the Mikro-Samplmill and in the hammer mill equipped with screens having 0.020- and 0.033-in. openings, respectively. The temperature of the product was kept below 122°F. (50°C.) by feeding ground, solid carbon dioxide with the dry gluten into both mills or by putting solid carbon dioxide in a jacket placed around the Mikro-Samplmill. When screens having 0.042-in. or larger openings were used, the product remained cool and about 90% of the particles passed through a 150-mesh sieve.

Baking Quality of Gluten from Different Flours. Drum-dried glutens for baking tests were prepared from eight different types of flour having wide ranges of physical and chemical characteristics and ranging in grade from patent flour to high-ash second-clear flour (Table V). The baking quality scores of vacuum-dried and drum-dried glutens separated from these flours are listed in Table VI. All vacuum-dried glutens and six of the drum-dried glutens exhibited good baking

TABLE V

PREPARATION OF DRUM-DRIED GLUTEN FROM VARIOUS FLOURS

Flour			Dispersion [a]		Drum-Dried Gluten		
Grade	Ash	Protein	HAc Used, Percent of Dry Gluten	pH	Total Protein	Rehydration Test	Nitrogen Soluble in 0.1*N* HAc
	% by wt (d b)	*% by wt (d b)*	*%*		*% by wt (d b)*	*arb. units*	*%*
Patent (HRW)	0.41	14.3	2.3	4.6	85.7	4 [b]	97 [b]
Straight (HRS)	0.41	17.2	1.5	4.8	78.8	3½	92
Second clear							
A	0.84	16.3	2.5	4.6	78.4	3½	93
B	1.26	16.9	3.0	4.6	75.4	3½	94
C	1.26	16.1	2.5	4.6	75.0	3	92
D	1.27	17.9	4.0	4.6	73.4	3	88
E	1.89	19.8	7.5	4.3	73.9	2½	85
F	1.95	19.8	7.5	4.3	71.0	1	78

[a] Dispersions contained 16.5% by wt. solids.
[b] Soluble nitrogen 97% and rehydration value 4 for vacuum-dried glutens.

qualities, but the baking quality decreased with increase in the ash content of the flour. Best results were obtained with glutens from the patent and straight flours.

Although most drum-dried glutens exhibited good baking qualities, they were somewhat below those of the corresponding vacuum-dried controls. The volume score was chiefly responsible for the low-

TABLE VI

BAKING QUALITY OF DRUM-DRIED GLUTEN FROM VARIOUS FLOURS

GLUTEN USED FOR FORTIFICATION	ABSORPTION	LOAF VOLUME[a]		VOLUME (15)		CRUST COLOR (5)		SYMMETRY (5)		EVENNESS (5)		TEXTURE (15)		GRAIN (10)		CRUMB COLOR (10)		AROMA (15)		TASTE (20)		TOTAL SCORE (100)	
		DD[b]	VD[c]	DD	VD	DD	VD	DD	VD	DD	VD	DD	VD	DD	VD	DD	VD	DD	VD	DD	VD	DD	VD
	%	*ml*	*ml*																				
Patent (HRW)	72.0	960	1075	10	14	4	4	4	4	4	4	13	13	8	6	9	9	13	13	18	18	83	85
Straight (HRS)	76.0	920	985	8	11	4	4	4	4	4	4	12	13	9	9	9	9	14	14	18	18	82	86
Second clear																							
A	76.0	830	945	5	9	4	4	4	4	4	4	11	13	8	9	7	8	14	14	18	18	75	83
B	74.7	855	950	6	9	4	4	4	4	4	4	12	13	9	9	8	8	14	14	18	18	79	83
C	75.5	905	905	8	8	4	4	4	4	4	4	12	12	8	9	9	8	14	14	18	18	81	81
D	74.7	860	950	6	9	4	4	4	4	4	4	12	12	8	8	9	8	14	14	18	18	79	81
E	74.7	690	920	0	8	4	4	4	4	4	4	9	11	6	7	6	8	14	14	18	18	65	78
F	74.5	690	890	0	7	4	4	4	4	4	4	9	12	6	8	6	8	14	14	18	18	65	79

[a] Loaf volume from standard white flour without fortification, 780 ml.
[b] Drum-dried.
[c] Vacuum-dried.

ered total baking score. Drum-dried glutens from low-ash flour were superior to similar products from high-ash flour. More acid was required to disperse gluten from high-ash flour, and in the case of flours with very high ash content (1.9%), a low-grade product resulted regardless of the amount of acid used.

Drum-dried products of excellent quality can be produced from wet wheat gluten separated from low-ash flours. Good-quality vital products can be made from wet gluten separated from other types of flour except those of very high ash content. The quality of drum-dried gluten from high-ash flours could usually be improved by using a lower solids content or pH of the dispersion, or by increasing the protein content (dry basis) of the wet gluten by better washing during separation.

Optimum Process Conditions

Wet wheat gluten from low-ash flours can be drum-dried commercially to a vital product after batch or continuous dispersion. Gluten premixed in a solution of acetic acid in water from an ion-exchanger (hydrogen cycle) can be dispersed by a Morden Stock Maker or a similar disperser equipped with a stator and adjustable rotor. Power required for such equipment, which imparts vigorous shear and impact action to the gluten in a short time, is approximately 125 watt-hours per lb. of dry gluten for a dispersing cycle of 15 minutes on a suspension containing 16.5% of gluten solids.

This dispersion is fed to a double drum-dryer at a pH ranging from 4.3 to 5.1. The optimum conditions under which 1 lb. of dry gluten per hour per sq. ft. of drying area is obtained are: a drying temperature about 260°F. (127°C.), a rotational speed of the drums about 12 r.p.m. or higher, and a roll clearance determined by the dryness desired. The dry vital gluten is pulverized to a suitable size in an attrition mill or in a hammer mill having a screen with 0.0625-in. holes.

The conditions used for producing vital gluten in the pilot plant can easily be adapted in commercial plants equipped with standard drum dryers, out it would be necessary to obtain suitable stainless-steel equipment for preparing the dispersion. The dried product should be entirely suitable for food uses or for preparing industrial chemicals.

Costs

A cost estimate has been prepared for a plant producing 6 million pounds annually of vital gluten with about 6% moisture by the proc-

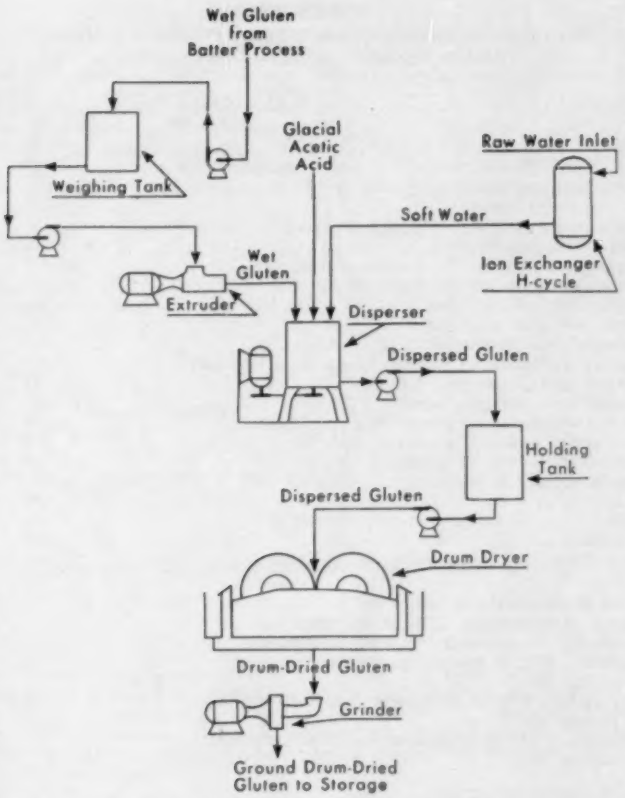

Fig. 2. Flow sheet for production of drum-dried gluten.

ess described. Assumptions made are that the vital gluten plant operates 20 hours per day for 250 days a year and is operated in conjunction with a plant from which wet gluten could be obtained. A flow sheet on which the cost estimate is based is shown in Fig 2. Table VII lists the land, building, and equipment required; the fixed capital investment is $477,000. Stainless-steel equipment is specified where necessary, and drum-dryers must be equipped with chrome-plated cast-iron rolls. Steam-generating facilities are not included as part of the equipment, but a charge for the steam utilized has been included in production costs. Table VIII shows the estimated drying cost to be 4 cents per pound of dry material when the plant is operated with a dryer capacity of 1 lb. of dried gluten per hour per sq. ft. of roll surface. This figure does not include the cost of the wet gluten, working

TABLE VII

FIXED CAPITAL INVESTMENT FOR A PLANT PRODUCING 6 MILLION POUNDS ANNUALLY OF DRUM-DRIED GLUTEN

ITEM		COST
Land	$ 1,000	
Building (75 by 65 by 12 ft.)	44,000	
Building improvements	7,000	
Total building cost		$ 52,000
Equipment, delivered:		
1 Cation exchanger, 6 cu. ft., high-capacity resin (30 kg/cu. ft.), S.S.	$ 2,800	
4 Drum-dryers, 300 sq. ft., chrome-plated rolls	144,000	
2 Dispersing units, 350 gal/hour, 25 hp., S.S.	20,000	
2 Tanks, 200 gal., S.S.-clad	1,700	
1 Tank, 600 gal., S.S.-clad	2,000	
1 Extruder, 150 lb/minute, 5 hp.	700	
3 Positive displacement pumps, 75 g.p.m., 5 hp., S.S.	9,000	
1 Storage tank, 2,000-gal., S.S. clad	3,500	
1 Storage tank, 100-gal., steel	150	
1 Positive displacement pump, 30 g.p.m., 3 hp., S.S.	2,000	
1 Centrifugal pump, 5 g.p.m., ¼ hp., S.S.	300	
1 Centrifugal pump, 50 g.p.m., 2 hp., S.S.	1,300	
1 Hammer mill, 50 hp.	5,000	
1 Scale	400	
1 Scale	150	
Conveyors	4,000	
Storage bins	4,000	
Bagger	2,000	
Total equipment cost, delivered		$203,000
Installation of equipment, 25% of delivered cost		50,750
Piping, wiring, instrumentation, 25% of installed cost		63,450
Contingencies, 15% of equipment installed complete with piping, etc.		47,600
Engineering fees, 15% of equipment installed complete with piping, etc.		47,600
Contractors fees, 4% of equipment installed complete with piping, etc.		12,600
Fixed capital investment		$477,000

TABLE VIII

ESTIMATED COST TO PRODUCE 6 MILLION POUNDS ANNUALLY OF DRUM-DRIED VITAL GLUTEN

(Basis 250 operating days per year, 20 hours per day)

ITEM		COST	
		Per Year	Per Pound
Raw material:			
Glacial acetic acid, 222,200 lb., 10 cents/lb.	$22,220		
Sulfuric acid 66° Bé, 9,000 lb., $2.25 per 100 lb.	203		
		$ 22,423	0.0037
Utilities:			
Water 2,031,000 gal. at 7.5 cents/1,000 gal.	$ 153		
Steam 39,771,000 lb. at 80 cents/1,000 lb.	31,817		
Power 1,569,290 kw.-hr. at 1.5 cents/kw.-hr.	25,539		
		$ 55,509	0.0093

(Continued)

TABLE VIII *(Continued)*

Item		Cost	
		Per Year	Per Pound
Labor and supervision:			
3 Men for dispersing, $2.50/hour	$15,000		
3 Men for drum-drying, $2.50/hour	15,000		
3 Men for drum-dryer helper, $2.25/hour	12,000		
3 Men for grinding and bagging, $2.50/hour	15,000		
1 Foreman	5,500		
1 Superintendent $10,000/year, ½ time	5,000		
Overhead	10,125		
		$ 77,625	0.0129
Maintenance:			
Equipment, 5%/year	$21,250		
Building, 2%/year	1,040		
		$ 22,290	0.0037
Fixed charges:			
Depreciation: Building 3%/year	$ 1,530		
Equipment 10%/year	42,500		
Taxes and insurance, 3%/year	14,310		
		$ 58,340	0.0098
Miscellaneous factory supplies and expenses		5,000	0.0008
Total		$241,187	0.0402

capital charges, selling and administrative expenses, interest, income tax, or profit.

Acknowledgment

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THE RELATION OF PARTICLE SIZE TO CERTAIN FLOUR CHARACTERISTICS[1]

BETTY SULLIVAN, W. E. ENGEBRETSON, AND MERLIN L. ANDERSON

ABSTRACT

A hard-wheat, 90% patent flour was air-classified in six particle size ranges and the separated fractions analyzed for particle size distribution, ash, protein, maltose value, gassing power, and viscosity as measured by the amylograph. The analysis of different samples of the same flour did not vary as greatly as does the analysis of the different size particles, themselves, that comprise the given sample of flour.

Both ash and protein content showed marked fluctuations with particle size ranges. At a very low micron size, the ash content was, roughly, double that of the original flour.

Ash decreased rapidly to about 15 microns where it was well below the original ash content. From 15 to 30 microns, the ash content increased, probably because the ratio of small endosperm cells and peripheral cells to more-or-less free starch granules increases. From 30 to 70 microns ash content again decreased, followed by an increase above 70 microns because there is a greater number of covered cells and aggregates in this size range.

Protein content followed the same general pattern, showing a wide range from 5.5% in the region around 15 to 30 microns (where free starch granules occurred in larger amounts) to well over 25.0% in the region under 5 microns.

The exact size at which these fluctuations in analysis occur and their extent depend on the wheat, the grade of flour milled from it, and the milling process used.

Maltose value and gassing power decreased with increasing particle size to approximately 70 microns where there was a definite increase.

Specific surface, which can be calculated from the particle size distribution curve, correlates very well with maltose value and gassing power for most flours – in fact, better than starch damage as it is presently measured. Starch damage is dependent on the type of grinding and does not necessarily parallel fineness of grind. Maltose values showed a log linear relationship with specific surface for a flour ground to varying degrees of fineness. The ratio of maltose value to gassing power increases as the particle size decreases.

Viscosity as measured by the amylograph showed an increase from the very small micron range to a peak around 20 to 30 microns, where there was the greatest concentration of free starch and the lowest protein content. There was then a drop in the curve, followed by an increase in viscosity in the coarsest size range.

Further work on the structure of the endosperm in relation to milling practices should lead to a better basis for assembling flour streams and to more realistic flour specifications.

Ash and protein content and some measure of malt response, such as maltose value, gassing power, or viscosity, are routinely used in

[1] Manuscript received August 10, 1959. Contribution from the Research Laboratories of the Russell-Miller Milling Co., Minneapolis, Minn.

the evaluation of flours. The relation of these factors to particle size and of particle size to the particular endosperm structure has not been sufficiently understood. Much excellent work on the structure of the wheat kernel is available and is beginning to receive the attention it deserves. As long ago as 1905, Cobb (10,11) published results on a comparative study of flour cells. He found a gradual decrease in the size of the starch granules from the center to the periphery of the endosperm. An exception is that part of the kernel near the crease, more particularly near the tip of the kernel where comparatively large starch granules occur, even near the outermost edge of the endosperm. Among the several descriptions of the general structure of wheat or its endosperm are those reported by Alexandrov and Alexandrova (1), Berliner and Rüter (3,4), Fairclough (14), Hayward (19), Percival (33), Vogel (37), and Winton and Winton (43). Greer and Hinton (15), Greer, Hinton, Jones, and Kent (16), Hinton (22,23), Hinton, Peers, and Shaw (24), and Jones (25), among others at the Cereals Research Station of the Research Association of British Flour-Millers, have made notable contributions to our knowledge of the structure of wheat and, in particular, the composition and distribution of certain constituents in the various dissected parts. In an outstanding series of papers, Bradbury, MacMasters, and Cull (5,6,7,8) reviewed earlier work and provided additional information on the gross anatomy and microscopic structure of various parts of the wheat kernel. Hess (20) and Hess and Mahl (21) have explored a new viewpoint of the structure of the endosperm and the formation of gluten by means of the electron microscope and X-ray and by ultraviolet fluorescent microscopic techniques. Two types of endosperm protein were separated by specific gravity using sedimentation in nonaqueous medium. Hess and co-workers termed these fractions "wedge protein" and "adhering protein" and reported them to be widely different in properties. According to Hess, only "wedge" protein forms gluten. Small amounts, a few percent or less, of wedge or "free" protein can be separated by sedimentation or air elutriation from a normal flour. More can be separated from a soft wheat flour than from a hard wheat flour and more from a finely ground flour than one milled conventionally. Elias and Scott (13), Hanssen and Niemann (18), and Wichser (42) have given some figures on the amounts and analyses of various fractions separated by air-classification of flour from hard and soft wheats. More recently Jones, Halton, and Stevens (26) have described flours obtained by the air-classification of hard and soft wheat flours.

For many years it has been recognized that the ash and protein

contents of flour are increased by small bran and germ particles that are occluded with the endosperm particles. It is less well known that cell-wall material from broken endosperm cells may contribute significantly to the ash content, particularly when it is present in higher than normal amounts. For example, when a fine, high-protein portion is air-separated from hard wheat flour, the ash is distinctly higher than the same particle size fraction of the same protein content separated from soft wheat flour. This is due to the greater amount of small broken pieces of cell-wall material in the hard wheat endosperm which, being small in size and light in density, are separated in the fine fraction. The cell-wall material has an important effect on viscosity, dough characteristics, color, and baking quality. About 65 to 70% of the endosperm cells are intact in a conventionally milled flour. This figure varies with the wheat variety, the tempering process, and the extent of grinding. Kent and Jones (27) gave an estimate of 65% intact cells in a straight-grade flour milled from Manitoba wheat, of which 13% were uncovered, 26% partly covered, and 26% covered; the balance of nonintact cells were given as 14% solitary and 21% aggregated. Flour is made up of peripheral, prismatic, and central endosperm cells which vary in size and shape. These cells contain large lenticular and small, more spherical starch granules, with some of intermediate size. The range in size of starch granules is from 1 to 50 microns. There are also present in flour very small percentages of free protein and endosperm cell-wall material from broken cells. The relative percentages of the different types of particles do not vary to any great extent from flour to flour of the same type, although marked variations occur in separate streams. The analysis of different samples of the same grade of flour does not vary as greatly as does the analysis of the different size particles, themselves, that make up the given sample of flour.

The uniformity of flour has assumed steadily greater importance with the continuing mechanization and control of the breadmaking process. The enzyme response of flour is a critical factor in maintaining this uniformity. Most bread flour milled from wheat grown in this country needs malt supplementation. Millers may add either wheat or barley malt flour. Bakers may use additional malt extract and various fungal enzymes. It has long been known that the response of flour to such enzymes depends on native starch susceptibility, the extent of starch damage, the level and balance of various enzymes of the flour and supplement, and the inactivation temperature of the enzymes. Sound, normal flours are considered by most investigators to contain largely beta-amylase with little, if any, alpha-amylase

except when milled from sprouted wheat.

There are several methods of measuring the malt response of wheat flour. These are the familiar maltose figure involving autolytic production of reducing sugars, gassing power in the 4th, 5th, or 6th hour, and the amylograph. In Europe where sprouted wheat is more of a problem, Molin's (30) method for the determination of sprout damage and its modification by Kent-Jones and Amos (28) as a dextrin figure are widely used. Each of these methods has certain advantages. The subject has been discussed by Dadswell and Gardner (12), Kent-Jones and Amos (28), and Sandstedt, Blish, Mecham, and Bode (35).

Any of these tests can be correlated with results of any baking test, but each method leaves much to be desired. While there is reasonable correlation between maltose, gassing power, and amylograph figures on the same grade of flour milled in one mill, correlations between any two of these measurements are not good in a comparison of flours milled from widely varying wheat mixes, of different grades, or with significantly different mill flows.

It has long been known that flours milled from certain wheats show poor malt response. Overgrinding of a flour, as for instance in a ball mill, will increase the response to malt because of smaller particle size, with consequent greater specific surface, and because of a large increase in damaged starch granules. However, the effect of particle size on malt response in a normal flour has not been clear; neither has the measurement of malt response to starch damage. Beta-amylase cannot act on raw, undamaged starch and it acts in flour only on those granules rendered susceptible to attack by other enzymes or by mechanical damage. The effect of excess grinding on the malt response and the baking quality of flour is usually attributed to starch damage, but our methods for measuring starch damage and consequent proof of this thesis leave much to be desired.

The studies reported in this paper were made to investigate the relationship of particle size and endosperm structure to ash, protein, maltose value, and gassing power as determined from air-classified fractions of a given flour. The effect of grinding on maltose value and gassing power and the dependence of these two measurements on the specific surface and starch damage will be discussed.

Materials and Methods

An untreated 90% patent flour milled from hard winter wheat, analyzing 0.42% ash and 11.2% protein on a 14% moisture basis, was used as a source material. It was air-classified in an Alpine 132-

MP Mikroplex Classifier nineteen times in order to make six fractions. A minimum of five classifications is necessary to make six fractions. The additional classifications may lead one to believe that excessive reduction of particles occurred. However, this was not the case; experience with this equipment has shown that most of the reduction is done during the first two or three classifications.

The aim in the present work was to resolve a flour completely into fractions of varying particle size by means of air-classification. In the coarser part of the range (above about 40 microns), this presented the difficulty that the range of separation is above that for

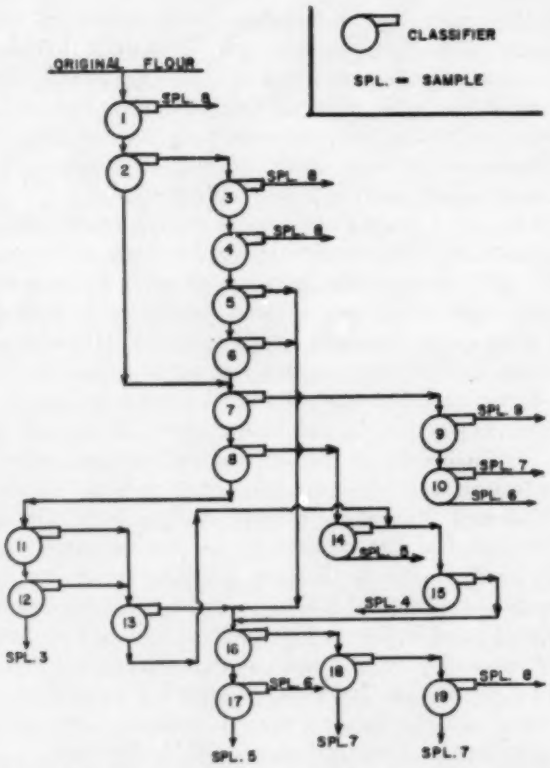

Fig. 1. Classification procedure. The vane setting is given in degrees and the feed setting in mm. They are as follows: 1) 20°, 25 mm.; 2) 50°, 30 mm.; 3) 20°, 25 mm.; 4) 25°, 20 mm.; 5) 50°, 30 mm.; 6) 30°, 30 mm.; 7) 40°, 25 mm.; 8) 45°, 25 mm.; 9) 25°, 20 mm.; 10) 50°, 25 mm.; 11) 50°, 25 mm.; 12) 50°, 25 mm.; 13) 50°, 25 mm.; 14) 50°, 30 mm.; 15) 50°, 30 mm.; 16) 50°, 25 mm.; 17) 50°, 25 mm.; 18) 50°, 20 mm.; 19) 50°, 20 mm. Feed rates varied between 80 lb. per hour and 400 lb. per hour.

which the Mikroplex Classifier is designed and, to obtain the desired cut size, it was necessary to feed the machine at relatively heavy rates in this range. With the Mikroplex, the actual cut size, obtained at any setting of the cut-size regulators, increases as the feed rate is increased. On the other hand, at the same time the sharpness of separation deteriorates. For this reason, a considerable number of classifications was necessary and, even so, the fractions finally obtained were not sharply defined in respect to particle size. Therefore, the chemical compositions of the particles theoretically present between various selected size limits were calculated from the data obtained on the experimental fractions.

The operation of the Alpine Mikroplex Centrifugal Air Classifier is described in detail by Rumpf and Kaiser (34). Briefly, the principle is as follows. A spiral air flow is induced toward the center of a shallow cylindrical chamber, the flat walls of which rotate at high speed to reduce drag effects. The sample to be classified is introduced into the chamber, whereupon each particle becomes subjected to two opposing forces: (a) a centrifugal force proportional to the cube of its size and (b) the air drag, proportional to the square of its size. For particles larger than the equilibrium or cut size (which is defined by the condition of operation), (a) will be greater than (b) and, for smaller particles, vice-versa. The coarse fraction thus accumulates in the peripheral region of the chamber, from where it is removed by a high-speed worm, whereas the fine fraction travels in the air stream to an exit at the center of the chamber and may be subsequently recovered by means of a cyclone or cloth filter. The operating cut size may be varied by changing the pitch of the spiral air stream by means of adjustable vanes through which the air passes on entering the separating chamber. The feed rate also affects cut size, but, at high feed rates, the sharpness of separation is reduced. A diagram of the classification procedure is shown in Fig. 1. The six samples of varying particle size ranges, from coarse to fine, are labeled samples 3 through 8.

The original flour was passed through the smooth rolls of an experimental Allis mill a total of five times in order to reduce the flour to a finer average granulation by roll pressure. This flour is labeled sample 1. The original flour was also ground in an Alpine 160-Z pin mill once at a speed of 17,500 r.p.m. This flour is labeled sample 2. Samples 1 and 2 were ground mainly for comparisons of maltose, gassing power, and amylograph data with the original flour.

The original flour, the ground samples, and the classified samples of varying particle size range were analyzed for ash, protein, maltose

value, and gassing power (6th hour) by conventional procedures as outlined in *Cereal Laboratory Methods* (2). Amylograph tests were made using 75 g. of flour at 14.0% moisture basis, 46 ml. of disodium phosphate citric acid buffer, and 414 ml. of distilled water (pH 5.35). The particular sample weight was chosen so all the determinations would be on the graph and as near as possible to the center. The particle size distribution was determined by the sedimentation method of Whitby (40,41). Whitby (40,41) and Cadle (9) have discussed the limitations of the sedimentation and other measurements of particle size distribution.

Microscopic examination of various fractions gave valuable information concerning types of cells. Starch damage was evaluated by staining with Congo Red (25) and also by the increase in maltose value on addition of 100 mg. of beta-amylase (Wallerstein) per 5 g. of flour.

Solution of Simultaneous Equations. A glance at the particle size distribution of the classified fractions, illustrated in Fig. 3, shows that there is a range in particle size in each of the fractions. It is apparent that the range in particle size overlaps from fraction to fraction, even on repeated air separations; i.e., particles of a given size may appear in more than one fraction. Arbitrary size ranges were selected and the proportion of particles in each interval was determined from the size distribution curves of each fraction. Equations were then set up for each fraction as follows:

$$\sum_{i=1}^{6} A_{ij} X_i = P_j$$

where A_{ij} = proportion by weight in the i^{th} size interval for the j^{th} sample;

X_i = the average $\begin{Bmatrix} \text{ash} \\ \text{protein} \\ \text{maltose value} \\ \text{gassing power} \end{Bmatrix}$ of the particles in the i^{th} size interval;

P_j = the chemical analysis for $\begin{Bmatrix} \text{ash} \\ \text{protein} \\ \text{maltose value} \\ \text{gassing power} \end{Bmatrix}$ of the j^{th} sample.

A system of six simultaneous equations with six unknowns was thus formed for ash, protein, maltose value, and gassing power.

One selection of intervals and the corresponding proportion in

the given range, as taken from Fig. 3, are shown in the following example.

Sample No.	*Proportion in Range (Microns)*					
	0–10	*10–20*	*20–35*	*35–60*	*60–85*	*85–120*
3	0.0	0.0	0.0	0.08	0.46	0.46
4	0.0	0.0	0.0	0.38	0.52	0.10
5	0.0	0.0	0.07	0.57	0.36	0.0
6	0.0	0.14	0.43	0.43	0.0	0.0
7	0.0	0.32	0.62	0.06	0.0	0.0
8	0.44	0.43	0.13	0.0	0.0	0.0

For the ash content, as shown in Table I, the equations are as follows:

$$0.08x_4 + 0.46x_5 + 0.46x_6 = 0.33$$
$$0.38x_4 + 0.52x_5 + 0.10x_6 = 0.33$$
$$0.07x_3 + 0.57x_4 + 0.36x_5 = 0.36$$
$$0.14x_2 + 0.43x_3 + 0.43x_4 = 0.43$$
$$0.32x_2 + 0.62x_3 + 0.06x_4 = 0.41$$
$$0.44x_1 + 0.43x_2 + 0.13x_3 = 0.59$$

The solution to this set of equations is:

$$x_1 = 0.88 \qquad x_4 = 0.41$$
$$x_2 = 0.33 \qquad x_5 = 0.26$$
$$x_3 = 0.48 \qquad x_6 = 0.38$$

The set of equations for protein, maltose value, and gassing power were solved similarly. Six points were determined for each curve in this manner. To improve the accuracy of the curves, numerical integration was used and the curves were adjusted accordingly. Figures 4 through 6 illustrate the results.

An assumption is made in the solution of these equations that a given x is constant for all samples. However, there is usually some deviation from this constant. This deviation can be minimized by selection of intervals where the slope of the curve is small; i.e., if, for a given x, $a - \Delta < x_i < a + \Delta$ for the set of equations, then as Δ becomes small, the equations will become more exact.

Results and Discussion

Table I gives the analytical data for the original flour, the ground samples, and the classified fractions.

Figure 2 gives the granulation of the original flour and of the same flour ground on rolls (sample 1) and by a pin mill (sample 2). Figure 3 shows the particle size distribution of the classified fractions (samples 3 through 8).

Ash. Figure 4 illustrates the ash content of flour in relation to particle size, expressed as Stokes equivalent spherical diameter in microns.

At a very low micron size (under 8 microns), the ash content is, roughly, double that of the parent flour. This is due partly to cell-

TABLE I

ANALYSIS OF AIR-SEPARATED FRACTIONS

SAMPLE	PERCENT OF ORIGINAL	RANGE OF PARTICLES [a]	MOISTURE	ASH [b]	PROTEIN [b]	MALTOSE VALUE [b]	GASSING POWER	AMYLOGRAPH
	%	μ	%	%	%	*mg*	*mg Hg 6th hour*	*B.u.*
Original flour	100.0	11 – 96	12.7	0.42	11.20	204	430	680
1	100.0	9.8– 76	11.2	.42	11.20	225	440	630
2	100.0	3.8– 60	7.3	.42	11.20	255	492	570
3	15.1	51 –115	9.6	.33	11.30	112	278	865
4	11.3	38 – 99	8.8	.33	12.30	106	271	835
5	15.8	30 – 83	9.0	.36	13.00	116	290	825
6	14.1	12 – 62	9.2	.43	10.80	180	400	770
7	23.4	11.5– 45	9.1	.41	7.30	237	472	860
8	20.3	3.3– 28	8.4	0.59	15.20	418	640	315

[a] The range is given as the mass median diameter ± two standard deviations.
[b] At 14% moisture.

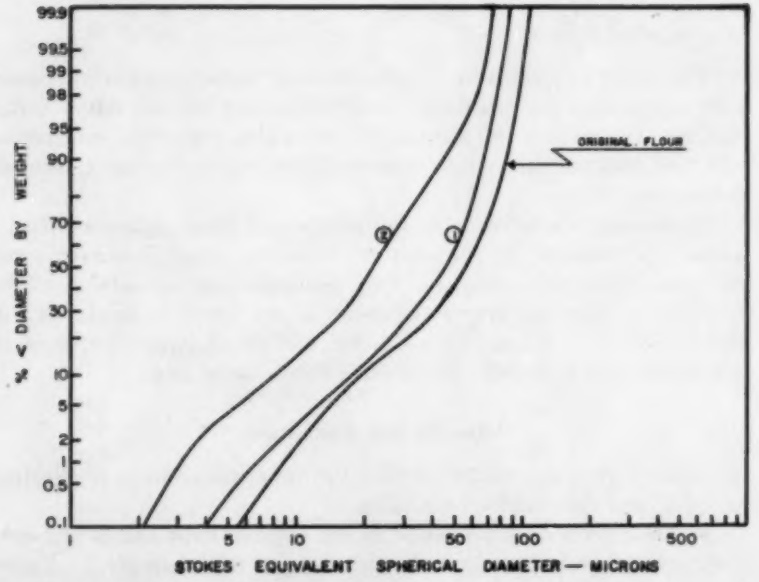

Fig. 2. Particle size distribution of original flour and of the same flour ground on rolls (sample 1) and by impact (sample 2).

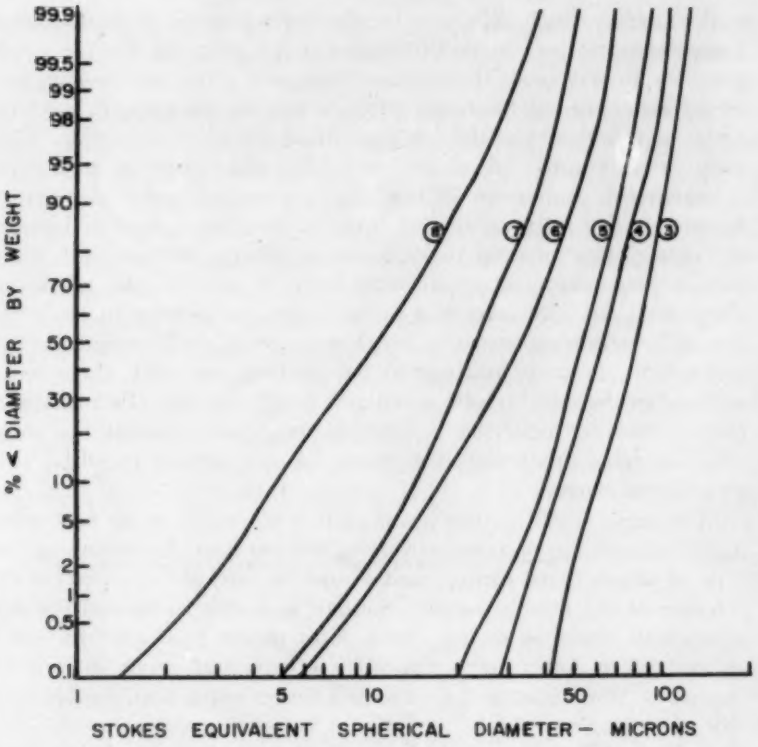

Fig. 3. Particle size distribution of classified fractions of flour.

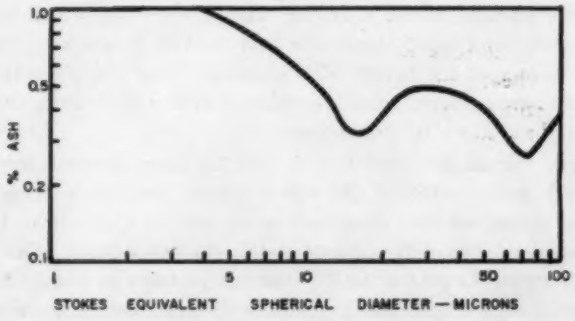

Fig. 4. Ash content of flour versus particle size.

wall material which separates in the finest fraction and also, to a lesser extent, to protein sheaths higher in ash than the smaller starch granules, to very small fragments of bran and germ, and to dust and crease dirt. The ash decreases precipitously to, roughly, 15 microns where it is well below the ash content of the original sample. This drop in ash content (from 0.42 to 0.30%) occurs because of the increase in the proportion of free starch granules in this size range. From 15 to 30 microns, the ash content increases, probably because the ratio of small endosperm cells and peripheral cells to starch granules increases. From approximately 30 to 70 microns, the ash again decreases; such a decrease may be related to an increase in the number of larger endosperm cells and a decrease of small, covered endosperm cells. From 70 microns to 100 microns and over, there is an increase in the number of covered and partly covered cells and aggregates containing higher-ash cell-wall material and probably also some aleurone cells; this probably accounts for the increase in ash in this particle size range.

The curve of ash content versus particle size varies in the maximum and minimum ash observed at varying micron sizes, depending on the type of wheat (hard spring, hard winter, or soft) and the percent extraction of the flour separated. Similar equations calculated for size separations made on spring wheat, short patent flour gave the same general curve, but a maximum ash is observed at about 50 microns instead of 30 microns as found with a longer extraction, hard winter wheat flour. The relative amounts of prismatic, central, peripheral, and aleurone cells vary with the grade of flour. Peripheral cells from winter wheat are reported to be smaller in size than from spring wheat. Endosperm cell-wall thickness and its relative ease of separation are important factors in the spectrum of ash versus particle size of flour. Larkin, MacMasters, and Rist (29), in examining Pacific Northwest wheats, found that the starchy endosperm cell walls near the aleurone layer were about one-half thicker (4 microns) than those near the center of the kernel (2.6 microns). The cell walls in the area next to the crease were from two to two and a half times thicker (7.3 microns) than those in the center.

Morris, Alexander, and Pascoe (31,32) have studied the distribution of ash and protein in the wheat kernel and, more recently, Hinton (23) measured the distribution of ash in the wheat kernel by means of hand-dissected parts of four different wheats. The aleurone layer accounted for 56.4 to 60.2% and the endosperm for 20.3 to 25.9% of the total ash. There was a gradient in ash content from the outer to the inner layers of the endosperm, except that the endosperm next

to the aleurone layer in the region of the crease was considerably higher. Hinton thought it probable that the gradient in ash content and in many other constituents throughout the endosperm is connected with the stage of development of the cells, increased concentration of ash, and decreased concentration of starch occurring in the less mature cells next to the aleurone layer.

Protein. The relation of particle size to protein distribution is shown in Fig. 5. As is well known, protein is highest in the smallest size range from 1 to 16 microns because more thin, light-density protein fragments are included in this fraction. The protein content decreases rapidly from 1 to 25 microns because a greater proportion of starch granules is included in the highest micron size. In fact, the fraction from about 15 to 35 microns is considerably below the protein content of the original flour, because this range includes an increased concentration of free starch granules.

The protein distribution curve rises steadily from 5.5 to 16.5% protein and from 25 to 45 microns as the ratio of more-or-less intact

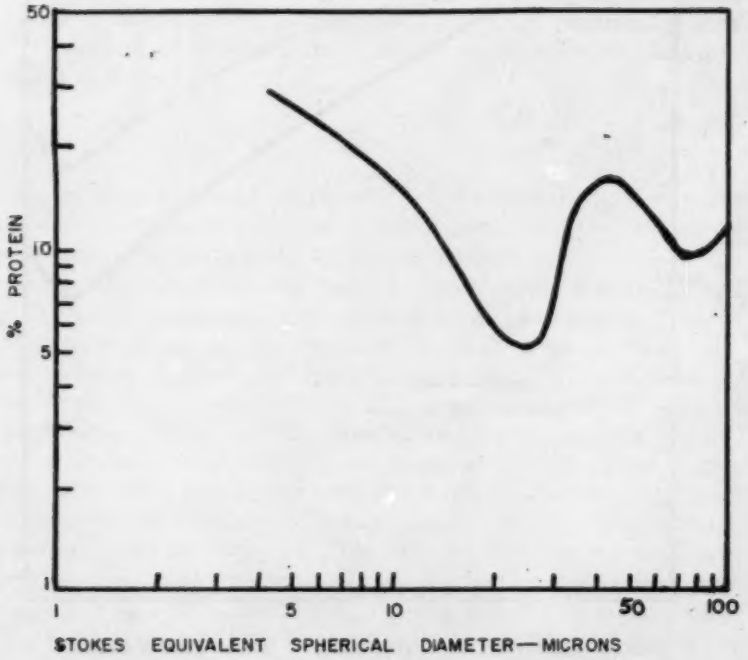

Fig. 5. Protein content of flour versus particle size.

endosperm cells to free starch granules increases. There is also an increase of prismatic and peripheral cells in this fraction. These cells are known to have a higher protein content than the central cells. From 45 to 70 microns, there is another, less sharp decline in protein percentage to somewhat below the protein content of the original flour. In this range, there are fewer peripheral cells and an increase in the number of central cells containing lower protein. More intact and aggregate cells appear in this range. Finally, from 70 to over 100 microns, there is another rise in protein content from, roughly, 9.0 to 12.0%. In this fraction, there is the highest concentration of covered and partly covered cells and double and multiple cells where no protein has been released which could account for the increase in protein content.

Maltose Value and Gassing Power. Figure 6 shows that the maltose value and gassing power of the particular flour used in these experiments are minimum at about 70 microns. Above and below this size,

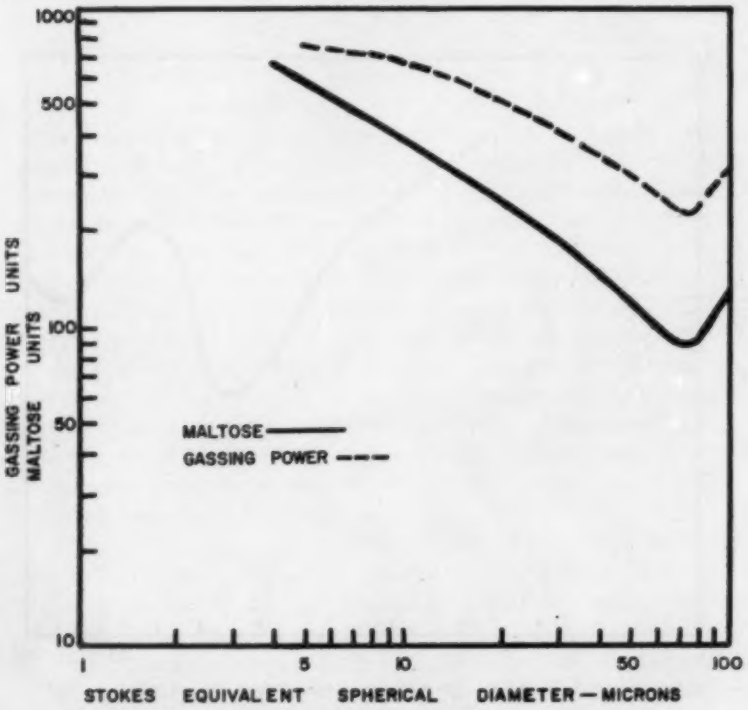

Fig. 6. Maltose value and gassing power of flour versus particle size.

maltose value and gassing power increase. For particles smaller than 70 microns, the maltose value and gassing power increase sharply with the decrease in particle size. It is difficult to account for the less marked, but decided, increase in maltose value and gassing power encountered in the size range coarser than 70 microns, since this fraction is made up of more intact endosperm cells and aggregates and has less specific surface and starch damage. A possible explanation might be that more amylolytic and/or cell-wall-splitting enzymes are present in these larger cells and aggregates. Some activity may be released during the course of the determination of maltose value and gassing power.

Similar calculations of simultaneous equations on the relationship of maltose value and gassing power to particle size on a short patent (80%) spring wheat flour gave a minimum point at 55 microns, instead of 70 microns as in the present study on a longer patent (90%) from winter wheat. The curves from both spring and winter wheat flours have the same general shape for both maltose value and gassing power.

For a given sample of flour ground to varying degrees of fineness, maltose value and gassing power show a log linear relationship with specific surface as calculated from particle size distribution. The formula is as follows:

$$A_t = 6 \sum \frac{f(d_i)_v}{d_i}$$

where A_t = total surface area per unit volume of particles; d_i = mean diameter of the particles in the i^{th} size interval; $f(d_i)_v$ = frequency by volume of the particles in the i^{th} size interval.

When flour is ground more finely, more endosperm cells are broken up, increasing the percentage of particles below 55 to 70 microns with consequent increase in specific surface. Normally, when ground by the same equipment, the finer the grind, the greater the starch damage. The shearing action and pressure of roll grinding, such as occur in conventional milling, produce more starch damage in achieving the same granulation than pin, stud, or fluid energy mills. Thus, in the hard winter wheat patent flour ground five times on smooth rolls (sample 1), there was more starch damage than in the same flour ground by impact in the Alpine 160-Z Pin Mill (sample 2). Yet the latter sample was considerably finer and showed a higher maltose value and gassing power. Microscopic examination of the ground flours, using 0.1% Congo Red solution, showed that, although "ghosts" were present in the pin-milled sample, there were virtually no cracked starch

granules; whereas, in the sample ground on smooth rolls, both radially damaged, cracked starch granules and more ghosts were present. Jones (25), in an excellent paper, stated that "granularity or particle size of flour and milling stocks is not for practical purposes a factor determining diastatic activity"; and further, that "in flours and intermediate stocks variously milled from a given wheat the maltose figure is a measure of the number of 'ghosts' present." Jones believed that the maltose figure is the result of the amount of damaged starch granules and the level of alpha- and beta-amylases. The amount of damaged starch is usually higher, the harder the wheat. When a given sample is ground in the same manner, as for example on rolls, the closer the roll setting and the greater the pressure, the finer the grind and the more starch-damaged granules, as Jones (25) has stated. However, in a comparison of stock ground by different actions, as for example rolls and impact or fluid energy, it is not valid to assume that starch damage necessarily parallels finer granulation or that particle size has no relation to maltose value.

Figure 7 illustrates the relationship of maltose value and gassing power with specific surface cm^2/cm^3. With any single conventionally

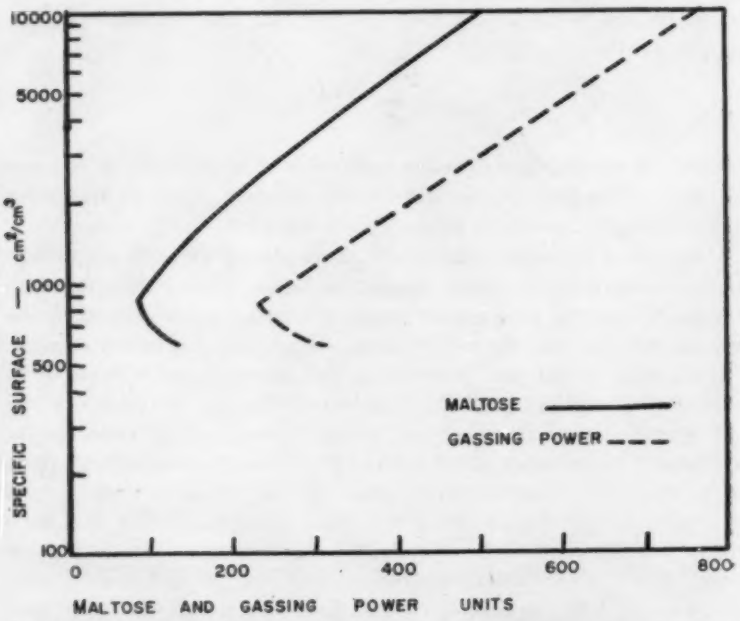

Fig. 7. Relation of specific surface of flour to maltose value and gassing power.

milled flour, there is a roughly linear relationship of maltose value and gassing power to specific surface, except in the region of lowest specific surface. For a given sample of flour ground to varying degrees of fineness, maltose value and gassing power show a high correlation with specific surface throughout the entire particle size range. This is because, in grinding more finely, more of the larger endosperm cells are broken open, thus increasing the percentage of smaller particles, the specific surface, and, consequently, the maltose value and gassing power.

Moreover, in our tests, it appears that both loaf volume and crumb resilience of bread flours are inversely correlated with specific surface.

The relation of starch damage to the specific surface and maltose value of flour needs further study. At present starch damage is usually measured by microscopic observation and counting of the damaged cells stained with Congo Red as originally proposed by Jones (25), by the amylose number advocated by Hampel (17), or by the increase in maltose value by the action of dialyzed malt extract (36). In our laboratory, we prefer to use the increase in maltose value with pure beta-amylase, together with microscopic observation of the stained cells. All of these methods leave much to be desired as quantitative procedures for starch damage. Measurements of increase in maltose value on addition of beta-amylase or dialyzed malt extract do not take into account how much damaged starch has been used as a substrate by the varying natural beta-amylases of flours.

It has been our experience that the larger starch cells (20 to 50 microns) are more subject to damage by roll pressure than the smaller cells that are present in the finest classified fraction. Yet the finest fraction is always very much higher in maltose value and gassing power than the coarser fractions. Since a damaged starch cell would be effectively lighter and its air drag increased, it would be found in a finer fraction than the normal starch granules of the same size. The relative influence on maltose value and gassing power of specific surface, of the concentration of damaged starch and of the amylases, themselves, in classified fractions remains to be determined.

Maltose value and gassing power increase in proportion to the specific surface only when the same type of grinding is employed. In an unclassified flour ground by different techniques, there is no significant change in total enzyme activity, but merely in specific surface and starch damage. The effect of two methods of grinding is illustrated in the following experiment.

A hard winter wheat patent flour, analyzing 0.39% ash and 11.5% protein (similar to but not identical with the flour that was classified),

was ground several times on closely set rolls and also on an Alpine 160-Z Impact Mill once at 17,500 r.p.m. and twice at 23,300 r.p.m. Maltose value was determined on each sample and on each sample plus 100 mg. beta-amylase. The difference in the two maltose values was taken as a rough measure of starch damage. Specific surface was calculated from the particle size distribution, as previously outlined.

TABLE II
EFFECT OF GRINDING ON MALTOSE VALUE

	Maltose Value	Difference in Maltose Value with Beta-Amylase	Specific Surface
			cm^2/cm^3
Original flour	158		
Original flour + beta-amylase	178	20	1,530
Roll-ground	586		
Roll-ground + beta-amylase	694	108	2,700
Impact-ground once, 17,500 r.p.m.	171		
Impact-ground + beta-amylase	196	25	3,170
Impact-ground twice, 23,300 r.p.m.	234		
Impact-ground + beta-amylase	290	56	5,320

Maltose value correlates with specific surface only when the same kind of grinding is used, as can be seen from the results in Table II. The flour severely ground on rolls showed more starch damage and gave a higher maltose value than the impact-ground samples of greater specific surface. The maltose value correlates with the specific surface on the two pin-milled samples as well as on flours ground on rolls.

There may be increased susceptibility to enzyme action by mechanical action of grinding in various ways that cannot be readily observed by presently used microscopic techniques. Reduction of particle size, by whatever means, is bound to result in stresses on the thin protein sheath surrounding the starch granules which would allow more ready access to enzymes. Moreover, internal stresses within the starch granule may cause rupture and cavitation on the granule surface, as Whistler, Goatley, and Spencer (38,39) have shown to result on the air-drying of corn starch. All of these points need further study.

Maltose Value Versus Gassing Power. There is reasonably good correlation between maltose value and gassing power. Many laboratories prepare curves showing a linear correlation of these two factors. But it is general experience that, with widely different mill mixes and with various flows and percentage extractions, decided departures from a straight-line relationship are common. Within a single milling or baking organization, the same chart cannot be used for all mills even when comparing the same grade of flour. The reason for this situation

is found in studying Fig. 6 on the relation of particle size to maltose value and gassing power. It is apparent that there is not a linear relationship and that the ratio of maltose value to gassing power increases as particle size decreases. Even for a given particle size, this ratio is not constant, but varies with the particular wheat mix, the milling practices, and the starch susceptibility.

Amylograph. No curve is shown for amylograph results versus particle size, since the relationship is more complicated than with the other analytical data. A few important points that have not had sufficient attention in considering amylograph data may be worthy of mention.

It is obvious that the higher the protein content of a flour, the less starch there is to gelatinize. Other factors being equal, the higher the protein content of a flour, the lower the amylograph viscosity. Beta-amylase has little effect on the amylograph figure of a normal flour. Table I shows that, on the unclassified samples, the finer the granulation, the lower the viscosity. The original flour showed 680 Brabender units as compared with 630 B.u. for roll-ground sample 1 and 570 B.u. for the more finely impact-ground sample 2. On the air-separated fractions which varied in protein content, the very fine fraction with the highest protein content (sample 8) showed only 315 Brabender units and the coarsest fraction (sample 3) gave 865 B.u. The intermediate classified samples followed no definite trend because of a number of factors, such as granulation and protein content, influencing the hot viscosity measurement. Viscosity figures do not lend themselves to treatment by simultaneous equations. The effect of particle size, protein content, and starch damage on amylograph figures will be discussed in another paper.

Further studies of the effect of milling practices on the structures of the endosperm and the relation of particle size and specific surface to various analytical specifications will lead to a sounder basis for the evaluation of flours.

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DIFFERENTIAL RESPONSE OF RICE STARCH GRANULES TO HEATING IN WATER AT 62°C.[1]

RUBY R. LITTLE AND GRACE B. HILDER[2]

ABSTRACT

When comminuted milled white rice was heated in water at 62°C. for 30 minutes and observed with a phase-contrast microscope, starch granules from 24 varieties were altered to various degrees. Starch underwent little or no change in most of the long-grain varieties, while that of most medium- and short-grain varieties was moderately to greatly altered. The heat-alteration values for lots grown in 1955 were highly correlated (−0.76) with taste-panel scores for cohesiveness for cooked samples from the same rice lots. Varieties likely to be cohesive when cooked may be tentatively classified by this test, for which only a few kernels of rice are required.

Studies on methods of evaluating and predicting the cooking characteristics of rice varieties have been conducted by the Human Nutrition Research Division (2,7). A wide range in texture was observed among varieties of milled white rice cooked by a standard procedure. Palatability evaluations of the texture of cooked rice require a minimum of 2 lb. of milled rice, carefully controlled cooking conditions, and a trained taste panel. Methods of predicting cooking behavior, utilizing objective measurements and requiring only a few kernels, have been needed. To supply basic information which might contribute to this end, microscopic studies of a number of varieties were undertaken.

Variation in rice quality is often attributed to some factor in starch properties such as amylose content (9,10) or gelatinization temperature. Although published reports of the gelatinization temperature of rice run from 61.2°C. (1) to 85°C. (11), a range of 24 degrees, such reports until recently have been based on rice of a single, often unspecified variety or commercially prepared starch, and the methods of determination were not uniform. The recently published article by Halick and Kelly (3) gives gelatinization temperatures from 58° to 79.5°C. for 18 rice varieties as determined by viscosity measurements.

To explore the possibility that the appearance of starch after heating in water under controlled conditions might be related to behavior of rice varieties during cooking, small samples of rice powder were ex-

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[2] Human Nutrition Research Division, Agricultural Research Service, U.S. Department of Agriculture, Washington 25, D.C.

amined microscopically after they were heated in water held at a constant temperature of 62°C. Microscopic appearance in phase-contrast illumination was employed as a measure of the alteration of starch granules. Percentages of granules unaltered to greatly altered were converted to heat-alteration values, which were found to be negatively correlated with taste-panel scores for cohesiveness or the tendency of cooked rice kernels to stick to each other. Steps in the heat-alteration process are illustrated in photomicrographs, and the differential responses are shown to be a direct indication of cooking quality of domestic varieties of milled white rice.

Materials and Methods

The 24 varieties of milled white rice examined were represented by one to six lots grown in 1953, 1954, and 1955, at several locations in the United States. Other experimental data, previously obtained in this laboratory on most of these lots, include water uptake ratio at 99°C., palatability evaluations by a panel (2), and differential response to dilute alkali (7).

Rice was prepared for the heat-treatment by two methods. In the first, 5 g. rice was soaked 1.5 hours in distilled water to soften the unusually hard kernels, ground coarsely while wet, dried, then further ground to 100-mesh size with a mortar and pestle. Damage, probably due to abrasion and enzyme action, was evident in about 25% of the starch granules from such preparations. In the second method, 5 g. rice was soaked 2 hours at 38–40°C. in 25 ml. 1% sodium chloride solution. The volume was brought to 195 ml. by adding cool 1% salt solution; the mixture was placed in a Waring Blendor, and the blender operated at medium speed for 15 minutes. The slurry was washed with 300 ml. distilled water while being filtered, then dried in air at room temperature. The second or blender method was preferred, since it involved less labor than grinding, caused little or no damage to starch granules, and resulted in a more finely divided flour without sieving. Flour was stored dry in stoppered vials in a refrigerator pending removal of aliquots for testing.

The standard method employed for heating samples consisted of stirring 0.25 g. of the rice powder 30 minutes in 65 ml. distilled water held at 62°C.[3] in a constant-temperature bath. Six replicate heating

[3] This temperature was chosen after preliminary treatments of flour from two varieties, Century Patna 231 and Caloro (a California lot), which had been rated widely different in cohesiveness (2) and in their response to dilute alkali (7). When observed in a polarizing or a phase-contrast microscope, starch from both varieties appeared birefringent or luminous and unaltered after treatment at 60°C., and nonbirefringent or completely darkened after treatment at 83°C. After treatment at 62°C., the starch from Century Patna 231 rice was unaltered (birefringent), while that from Caloro was greatly altered (nonbirefringent). The phase-contrast microscope alone was adopted for subsequent observations, since it offered two advantages over the polarizing microscope, namely visibility of stages in the heat-alteration process, and visibility of completely gelatinized starch granules.

treatments were performed for each lot, three using flour prepared by grinding and three using flour prepared by the blender method. Rice flour soaked 30 minutes in water at room temperature provided the controls. At the end of the heating period, a 2-ml. portion of the suspension was diluted to 15 ml. with water at room temperature. A drop of the dilution was placed on a slide, covered, drained of excess liquid, and sealed with petroleum jelly, using care to avoid pressure on the coverglass. Slides were examined microscopically with a Bausch & Lomb binocular research microscope with positive or dark contrast phase accessories and a magnification of 970 diameters. Each slide was moved from left to right or right to left by mechanical stage, and the first 100 granules passing through a specified portion of the field were classified in one of four categories. The classification was based primarily on degree of apparent darkening or loss of luminosity, although increased size usually accompanied darkening. Since the range in original diameters was rather wide[4] and the actual process of heat alteration was not followed visually, the exact magnitude of swelling could not be determined for individual granules. A comparison of unaltered with greatly altered granules in some of the easily affected varieties demonstrated that granules may expand little, or they may expand as much as four times in diameter (64 times in volume). The four categories, illustrated in Fig. 1, were characterized as follows:

1. Granules unaltered, angular, luminous, and without a definite hilum although often slightly darker in the center. Starch from all unheated samples was of this appearance[5], as was most starch from treated samples of some varieties.
2. Granules slightly altered, darkened, cracked, or striated from the center outward, but luminous in half or more of their diameter, perhaps slightly swollen. Granules of this type were found only in heated samples except as noted (see footnote 5).
3. Granules moderately altered, more than half darkened, usually swollen, with luminous areas in the form of blocklets, striations, or uniform or patterned rims or convolutions on the surface of the otherwise darkened structure. Moderately altered granules occurred only in heated samples.
4. Granules greatly altered, slightly to greatly swollen, lacking luminosity or completely darkened, but definitely outlined and surrounded by a lighter halo. Markings suggestive of those described for cate-

[4] Granule diameters of untreated rice starch ranged from 2 to 9 microns and averaged about 5 microns with no differences due to variety or grain length.

[5] Unheated or control samples from all varieties prepared by grinding showed 25% or more of the starch granules in categories other than No. 1, chiefly category 2, due to abrasion damage. Damaged granules were more easily altered by heating.

gory 3 were commonly present, but were darker than the major portion of the granule. Greatly altered granules were found only in heated samples.

After the distribution of 100 granules in the four classes had been tabulated, all granules of category 1 were given a weight of 1, those of category 2 a weight of 2, those of category 3 a weight of 3, and those of category 4 a weight of 4. The weighted values were totaled and divided by 100 to arrive at a "darkening index" for the replication. Darkening indexes of unheated replications, secured in the same manner, were deducted from darkening indexes of heated replications

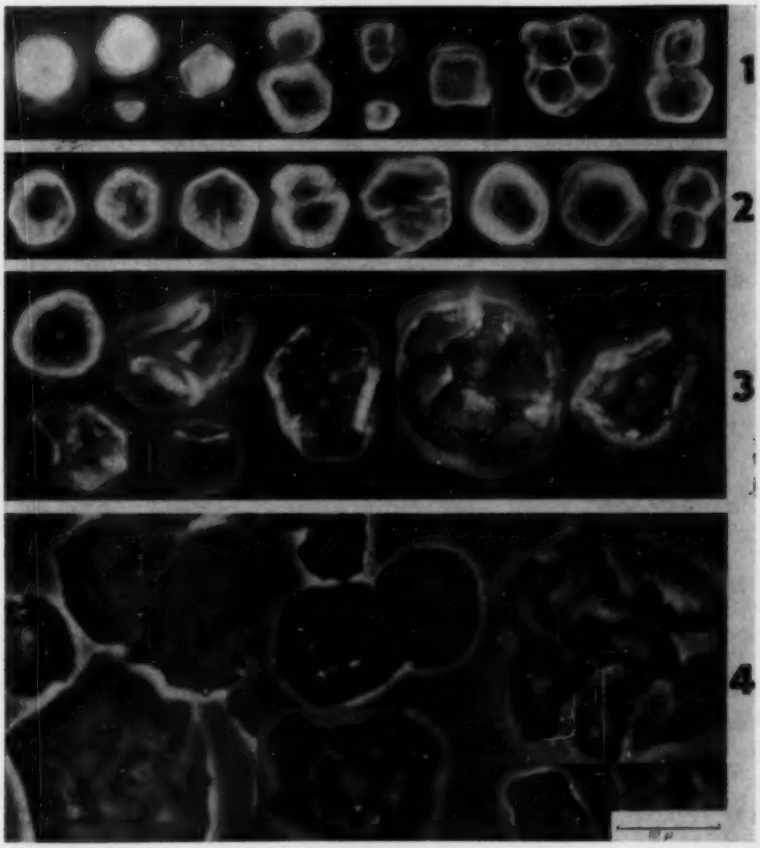

Fig. 1. Photomicrographs of rice starch granules as affected by heating 30 minutes at 62°C., illustrating four categories or degrees of alteration: 1, unaltered; 2, slightly altered; 3, moderately altered; 4, greatly altered.

to give corrected "heat-alteration values."

Statistical analyses indicated that good precision was obtained by the heat-alteration method. The standard deviation was 0.27; the standard error of the mean based on 18 replications of 100 granules each was 0.1 while that of the mean based on three replications was 0.2 (6). These statistical analyses were based on the observations of two persons; in some cases all replications for a lot of rice were classified by one observer; in other cases replications by both observers were included. As frequently happens when biological materials are being classified, some difficulty was experienced in classifying a few borderline cases between categories 1 and 2, but rarely between other adjacent categories. On the basis of statistical analysis, it was decided that in routine testing of samples prepared by the blender method, classification of granules in only one unheated control and two replications of the heating treatment would be sufficient.

The photomicrographs shown were made with a Bausch & Lomb Model N camera, 15× ocular, apochromatic oil-immersion phase-contrast objective, and Eastman Kodak 35-mm. triple-X film. Illumination was provided by a Nems-Clarke "Pulsarc" xenon arc lamp, which can be used at a standard intensity for viewing and focusing, and pulsed at four different elevated intensities expressed in milliseconds. The optimum exposure for showing detail varied from 40 milliseconds for unaltered granules to 135 milliseconds for greatly altered granules. The light level necessary for proper exposure of greatly altered granules in water mounts was too intense for unaltered granules. Therefore exposures were aimed at showing details such as the eye would see, rather than at illustrating the actual visible contrast between granules at the two ends of the scale.

Results and Discussion

Table I shows a summary by variety of 1) percentage distribution of granules in the four categories, 2) darkening indexes, and 3) heat-alteration values for samples prepared by the blender method.

The 24 rice varieties fell into three groups, based on heat-alteration values, roughly corresponding to the grain-length classification. Most starch granules from the long-grain rices, except in the varieties Rexark and Toro, were unaltered or slightly altered. Most of the medium-grain rices showed a high proportion of starch granules in the moderately altered condition; of the two exceptions, starch from Early Prolific was mostly unaltered and that of California-grown Calrose was greatly altered. In short-grain rices most starch granules were moderately altered, except for those lots of Caloro and Colusa grown

in California, in which a majority of the granules were greatly altered

Heat-alteration values for the lots grown in the year 1955 were compared statistically with taste-panel scores for cohesiveness for these lots (2) (Table II). When the data for all varieties were considered, a highly significant correlation coefficient of –0.76 was calculated. The regression curve obtained is shown in Fig. 2. When data were classified by grain length, the correlation coefficient for long-grain samples

TABLE I

ALTERATION OF STARCH GRANULES FROM DIFFERENT RICE VARIETIES AFTER HEATING 30 MINUTES AT 62°C.

VARIETY	No. OF LOTS	DISTRIBUTION OF GRANULES IN CATEGORIES[a]				DARKENING INDEX[b]		HEAT-ALTERATION VALUES[c]
		1	2	3	4	Heated	Controls	
		%	%	%	%			
LONG-GRAIN								
Bluebonnet	1	76	21	3	0	1.3	1.0	0.3
Bluebonnet 50	4	65	27	7	1	1.4	1.0	0.4
Century Patna 231	6	89	9	2	0	1.1	1.0	0.1
Fortuna	2	81	17	2	0	1.2	1.1	0.1
Improved Bluebonnet	2	78	19	3	0	1.3	1.2	0.1
Rexark	1	20	20	49	11	2.5	1.1	1.4
Rexoro	4	63	29	8	0	1.4	1.0	0.4
Sunbonnet	3	73	23	4	0	1.3	1.2	0.1
Texas Patna	3	75	21	4	0	1.3	1.2	0.1
Toro	3	13	11	54	22	2.8	1.1	1.7
Texas Patna 49	2	81	17	2	0	1.2	1.0	0.2
B4512A1-20	1	42	49	7	2	1.7	1.0	0.7
B4512A1-32	1	83	14	2	1	1.2	1.0	0.2
B455A1-25	2	76	20	4	0	1.3	1.1	0.2
MEDIUM-GRAIN								
Blue Rose	2	12	12	53	23	2.9	1.2	1.7
Calrose (Texas)	2	26	24	43	7	2.3	1.1	1.2
Calrose (Calif.)	1	0	0	49	51	3.5	1.0	2.5
Early Prolific	2	73	24	3	0	1.3	1.0	0.3
Magnolia	2	16	19	55	10	2.6	1.2	1.4
Nato	2	21	19	49	11	2.5	1.1	1.4
Zenith	5	21	12	43	24	2.7	1.1	1.6
SHORT-GRAIN								
California Pearl	1	0	0	24	76	3.8	1.0	2.8
Caloro (Texas)	4	17	17	47	19	2.7	1.1	1.6
Caloro (Calif.)	2	0	1	15	84	3.8	1.0	2.8
Colusa (Texas)	2	18	23	50	9	2.5	1.0	1.5
Colusa (Calif.)	1	0	1	10	89	3.9	1.0	2.9
11-47-11-1	2	5	8	59	28	3.1	1.1	2.0
12-47-6-2	2	28	24	38	10	2.3	1.1	1.2
Test difference[d]								0.5

[a] Code: 1, granule luminous, entire, unaltered; 2, granule luminous in more than half of diameter, darkened or cracked in center; 3, granule darkened in at least half of diameter, with luminous rim, striations or other markings; 4, granule completely darkened. Swelling usually accompanies darkening.

[b] Percentages multiplied by values of 1, 2, 3, or 4 for the respective categories totaled and divided by 100.

[c] Darkening index of heated samples minus darkening index of unheated samples.

[d] The difference between any two means is significant at the 5% level when it equals or exceeds the test difference.

TABLE II

HEAT-ALTERATION VALUES AND PANEL SCORES FOR COHESIVENESS FOR LOTS OF RICE GROWN IN 1955

VARIETY	HEAT-ALTERATION VALUES[a]	PANEL SCORES FOR COHESIVENESS[b]
Century Patna 231	0.1	6.0
Improved Bluebonnet	0.0	6.6
Texas Patna 49	0.2	6.2
B4512A1-32	0.2	6.5
Fortuna	0.2	5.6
B455A1-25	0.2	5.6
Early Prolific	0.2	5.3
Bluebonnet 50	0.4	6.1
Rexoro	0.4	6.6
Nato	1.2	4.4
12-47-6-2	1.2	4.3
Calrose (Texas)	1.3	4.8
Zenith	1.4	5.1
Magnolia	1.5	6.4
Colusa (Texas)	1.7	4.1
Toro	1.8	5.0
Caloro (Texas)	1.8	4.9
Blue Rose	1.9	4.5
11-47-11-1	2.1	5.2
Caloro (Calif.)	2.8	3.1

[a] See Table I.
[b] Based on scale of 9 (least cohesive) to 1 (most cohesive) (see reference 2).

was calculated as −0.73, which was significant at the 5% level. Correlations for the medium-grain and short-grain samples were not significant for the limited numbers of samples used in the calculations.

As a means of characterizing the rice varieties studied, the heat-alteration technique has yielded results generally consistent with those from other methods of evaluation (2,3,4,5,8,10), most of which indicate the importance of starch in determining the processing behavior of rice. Some varieties such as Rexark, Toro, and Early Prolific differ from others of their grain-length in this as in other respects, reaffirming that grain length is not a wholly reliable guide to the processing characteristics of a rice variety.

While gelatinization temperatures were not determined in this work, the evidence presented here seems to indicate that rice varieties differ in this respect. It was shown that the threshold temperature for complete darkening of starch granules as observed in the phase-contrast microscope was as low as 62°C. for some of the most cohesive rices. Since completely darkened or nonluminous starch also lacked birefringence in the polarizing microscope, it is suggested that such starch may be considered fully gelatinized microscopically. It is further suggested that loss of luminosity in positive phase contrast could serve as a criterion for determining gelatinization temperatures micro-

scopically. Varieties having high heat-alteration values thus may have low gelatinization temperatures, and varieties having low heat-alteration values may have high gelatinization temperatures. A comparison of the 14 varieties for which both heat-alteration values and gelatinization temperatures (3) are available lends support to this theory.

It is well known that not all starch granules from a sample gelatinize at the same temperature. Definite gelatinization temperatures are difficult to establish microscopically when loss of birefringence in polarized light is the criterion, since gelatinized granules are no longer visible and their presence may not be detected. In consideration of the results reported herein, use of the phase-contrast microscope with observations of granule alteration in water at 62°C. would seem to provide a useful means for objectively comparing rice varieties and differentiating types of rice having different cooking characteristics.

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EFFECTS OF FATS AND NONIONIC SURFACE-ACTIVE AGENTS ON STARCH PASTES[1]

ELIZABETH M. OSMAN AND MARION R. DIX

ABSTRACT

Studies with a Brabender Amylograph showed that viscosity increased in a 6% corn starch paste at a progressively lower temperature as fat was added. The eleven natural and hydrogenated fats used, although varying widely in degree of unsaturation, showed substantially no differences in their effects on the gelatinization or the cooling curves of the starch paste.

Addition of surface-active agents to the starch-water-fat mixture usually resulted in a marked increase in the temperature at which viscosity increased, as well as in the shape of the cooling curve. These effects appeared to be related to the length of the hydrocarbon chain and the number of hydrocarbon chains in the molecule of the surface-active agent. The character of the hydrophilic portion of the molecule also exerted an effect.

Little information has been reported concerning the effects of fats on the cooking of starch. This is true, also, for surface-active agents of the types used in foods as emulsifying agents or bread softeners. The few studies published have indicated that fat has little or no effect on gelatinization (5,11,12). Investigations of the action of monoglycerides (12) and polyoxyethylene monostearate (2,4,5,6,9) have shown that both these surface-active agents inhibit the swelling of the starch granules and cause starch gels containing them to be soft and easily broken. In the present study the Brabender Amylograph was used to secure more complete information on the effects of substances of these types on starch gelatinization, as reflected by viscosity changes, and to demonstrate the relationships between their chemical structures and actions on the starch pastes.

Materials and Methods

The following fats and oils (Iodine Numbers in parentheses) were used in 6% corn starch pastes in amounts varying from 0.2 to 12% of the weight of the starch-water mixture: soybean oil (132) and five hydrogenated soybean oils (105, 93, 82, 69, and 38), corn oil (126) and two hydrogenated corn oils (99 and 59), cottonseed oil (109), and prime steam lard (62). Total volume of the mixture was kept at approximately 400 ml., since with larger volumes the fat was not dispersed adequately. Thus, the ratio of 24.0 g. (d.b.) of starch to 376.0 g. of water was retained, but the actual amounts used were decreased as

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amount of fat added was increased from 0.2 to 12.0% of the sum of the weights of the starch and water.

The oil or melted fat was blended thoroughly with the starch and the resulting mixture was slurried in the water, which contained sufficient sodium hydroxide to produce a pH value of approximately 6.5 in the paste after gelatinization. This method of mixing gave much better dispersion of the fat in the cooked paste than addition of melted fat to a starch-water slurry. The mixture was heated in a Brabender Amylograph from 50° to 95°C., held at 95°C. for 15 minutes, then cooled to 30°C. and reheated to 95°C. For determination of gel strength with the Corn Industries Gelometer, a similar mixture was poured after the 15-minute holding period at 95°C. into the gelometer testing jars, and the disks were embedded. Samples were stored at approximately 2°C. for 18 hours, then allowed to stand at room temperature for 15 minutes to minimize effects of temperature change on gel strength, and the test was made.

For tests of the effects of surface-active agents on starch pastes, the basic mixture was a 6.5% corn starch paste to which soybean oil was added in an amount equal to 6% of the weight of the starch-water mixture. Sufficient sodium hydroxide was used to produce a pH value of approximately 6.5 in the paste after gelatinization. When a surface-active agent was used, it was substituted for 6% of the oil in the basic mixture (i.e., its weight was 0.36% that of the starch-water mixture), and the weight of oil was reduced by the same amount. The surface-active agent was dispersed in the oil. As in the studies of the effect of fats and oils alone, the oil mixture was then blended thoroughly with the starch and the resulting mixture slurried in the water. The slurry was heated in the amylograph from 50° to 96°C., held at 96°C. for 15 minutes, cooled to 30°C., reheated to 96°C., and recooled to 30°C. Gels were prepared from similar samples held at 96°C. for 15 minutes.

At least two preparations of each combination of ingredients were pasted, one for complete record of viscosity changes and the other for gel strength determination. In most cases the viscosity values at comparable pasting times agreed within 10 Brabender units (B.u.) and the temperatures at maximum viscosity within 1.0°C. In the few cases where agreement was not within 20 B. u. (the reported reproducibility of the instrument (3)), or 2.0°C., several additional samples were pasted, all of which produced results agreeing, within the limitations of the instrument, with one of the original values.

Results and Discussion

The effects of additions of increasing amounts of soybean oil to a

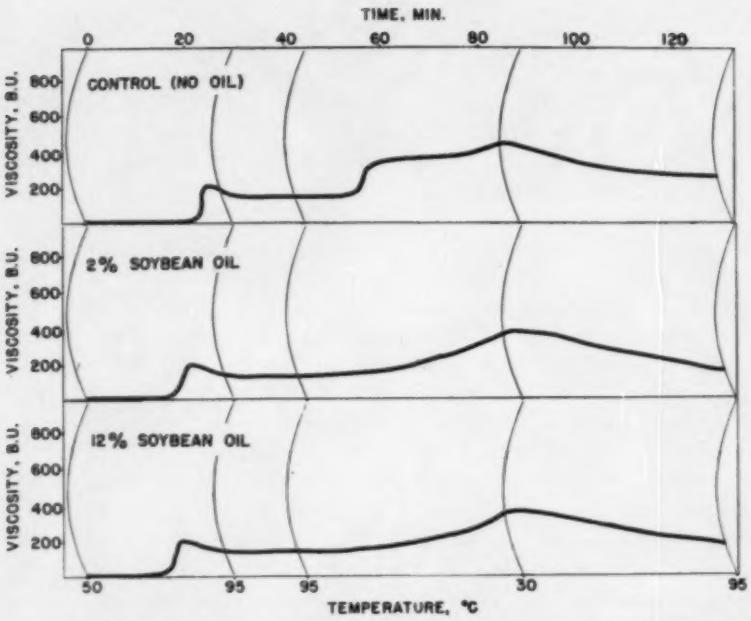

Fig. 1. Effects of soybean oil on amylograph curves of 6% corn starch pastes.

corn starch paste are demonstrated by the curves in Fig. 1. The addition of oil had no appreciable effect on the maximum value for hot-paste viscosity during the first heating. However, it appeared to lower slightly the temperature at which the initial increase in apparent viscosity was recorded, and it had a marked effect on the temperature at which maximum viscosity was reached.

As Anker and Geddes (1) have pointed out, the initial increase in viscosity is so gradual that the error in estimating the exact temperature at which it occurs, which they termed "temperature of transition," tends to be much higher than that involved in estimating the temperature at maximum viscosity. The temperature at the initial increase in viscosity lacks the significance of the gelatinization temperature, at which the starch granules lose their birefringence. Therefore, since the temperature at maximum viscosity appeared to have greater practical importance, as well as being subject to more accurate determination, it was the value chosen for making comparisons of the systems studied (Table I).

The effect of soybean oil in lowering the temperature at maximum viscosity became progressively greater as the amount was in-

TABLE I

EFFECT OF CONCENTRATION OF SOYBEAN OIL ON TEMPERATURE AT MAXIMUM VISCOSITY

CONCENTRATION, AS PERCENT BY WEIGHT OF 6% STARCH PASTE	TEMPERATURE AT MAXIMUM VISCOSITY
	°C
0.0	92
0.2	90
0.5	90
1.0	88
2.0	87
4.0	85
6.0	84
9.0	82
12.0	82

creased, until the weight of oil equaled 9 to 12% that of the starch-water mixture. Larger amounts of oil seemed to have little additional effect, probably because no more oil could be well dispersed in the mixture. In addition to lowering the temperature of maximum hot-paste viscosity, the oil removed the characteristic step found in the cooling curve of the corn starch control paste (Fig. 1). Even 0.2% soybean oil completely eliminated this step. The presence of the oil lowered the viscosity attained when the paste was cooled in the amylograph to 30°C., and also after it had been reheated to 95°C.

Gels prepared from samples removed after the 15-minute holding period at 95°C. did not appear to differ substantially from the control. The inconsistencies in gel strength values observed may have been partially the result of the inhomogeneity of the pastes.

Neither degree of saturation nor source of the fat appeared to make any difference in its effect on the starch paste except in the case of pastes containing the most saturated fat, hydrogenated soybean oil with an iodine value of 38. Pastes containing this fat, when cooled to 30°C., had lower viscosities than the other pastes at this temperature. This effect was possibly associated with the fact that the fat particles solidified. At room temperature this fat was very hard and brittle.

In marked contrast to the similarity of the various fats and oils in their effects on the amylograph curves were the wide variations in effects obtained with various nonionic surface-active agents. The compounds listed in Table II were substituted for part of the fat in a starch paste containing soybean oil. Use of a mixture of surface-active agent in oil rather than the surface-active agent alone appeared to have several distinct advantages:

1. The combination formed a system similar to that found in foods, in which fat is nearly always present when a surface-active agent is used.

2. Several surface-active agents, when used without fat or oil, raised the temperature of maximum viscosity to such an extent that it was not reached even when the final temperature was raised from 95° to 96°C. and the mixture was held at 96°C. for several minutes. As a result, the shapes of the cooling curves of the pastes were greatly influenced by whether or not maximum viscosity had been reached, as well as by other effects of the surface-active agents. By taking advantage of the effect of oil in lowering the temperature of maximum viscosity, and by heating the mixture to 96°C., a peak viscosity was reached during the heating cycle with all but one of the mixtures studied. The cooling curves of the resulting pastes showed characteristics which appeared to be related to the structures of the surface-active agents present.
3. Fat-containing pastes permitted a better comparison of the relative effects of the various surface-active agents on temperature at maximum viscosity, because of the range between the temperature at the peak viscosity for the control, 81°C., and the top of the heating cycle, 96°C.
4. When fat was not used, larger amounts of surface-active agents

TABLE II

EFFECT OF SURFACE-ACTIVE AGENTS ON STARCH PASTES CONTAINING FAT

Ester	Temperature at Maximum Viscosity	Gel Strength
	°C	g.-cm.
Glyceryl monopalmitate	94	186
Glyceryl monostearate	96	150
Methyl alpha-D-glucoside-6-laurate	75	105
Methyl alpha-D-glucoside-6-palmitate	88	78
Methyl alpha-D-glucoside-6-stearate	92	73
3-Palmitoyl-D-glucose	92	...
3-Stearoyl-D-glucose	½ minute at 96	62
Ascorbyl palmitate	96	123
Sorbitan monostearate	94	132
Sucrose monostearate	2 minutes at 96	91
Polyoxyethylene monostearate (MYRJ 45)	1 minute at 96	43
Polyoxyethylene monostearate (MYRJ 52)	no maximum	18
Sucrose dipalmitate	84	59
Sucrose distearate	90	58
Sucrose ester (Seqol 260)	87	84
Polyoxyethylene sorbitan monooleate	91	130
Polyoxyethylene sorbitan monostearate	92	118
Methyl glucoside distearate	89	85
Methyl glucoside tallow emulsifier No. 2210	90	105
Methyl glucoside tallow emulsifier No. 2275	90	107
Lecithin	77	443
Sorbitan tetrastearate	84	379
None (6.5% starch paste with 6% soybean oil)	81	608

appeared necessary to produce noticeable effects. In the quantities used, some of them caused foaming of the mixture or caking of the surface-active agent on the cooling coil. These difficulties were not encountered in the fat-containing systems used.

The magnitude of the maximum viscosity reached during heating remained substantially unchanged, but the temperature at which it occurred was influenced by the presence of surface-active agents, as might be predicted from published results on the effects of some of these compounds on the swelling of starch granules (2,5,12). The length and number of fatty acid residues, as well as the nature of the hydrophilic moiety in the molecule appeared to affect this phenomenon (Table II).

The temperature at which maximum viscosity occurred with monoesters was higher the longer the hydrocarbon chain. With glyceryl monostearate it was 96°C., and with glyceryl monopalmitate, 94°C. Methyl alpha-D-glucoside-6-palmitate did not raise the temperature as much as methyl alpha-D-glucoside-6-stearate; the corresponding laurate, instead of raising it, lowered it below that of the control paste. Likewise, 3-palmitoyl-D-glucose did not raise the temperature as much as 3-stearoyl-D-glucose. A similar relationship appeared to prevail with compounds containing more than one fatty acid residue; for example, sucrose distearate raised the temperature of maximum viscosity more than sucrose dipalmitate.

An increase in the number of fatty acid groups from one to two lowered the temperature at maximum viscosity in the case of the stearates of both sucrose and methyl alpha-D-glucoside. However, in neither case was the diester preparation a single chemical compound and this effect, therefore, must be viewed with reservation.

Comparison of the various monostearates and also of the monopalmitates indicated that the nature of the hydrophilic portion of the molecule had a decided effect on the temperature of maximum viscosity, but the number of compounds studied was too small to give an indication of the factors involved.

The shapes of the cooling curves obtained when various surface-active agents were used showed great differences. Most of the monoesters (Fig. 2) produced cooling curves with a peak at about 70°C., although the temperature varied somewhat from one ester to another. These peaks were repeated in a second cooling curve after the mixture had been heated a second time. They may be related to the characteristic step in the cooling curve of ordinary corn starch. The step could be removed from the curve of corn starch by "defatting" through alcohol extraction, as previous investigators (10) had observed, as well

as by addition of oil, as discussed above. Although it has been stated that the curve of defatted starch can be returned to its original form by addition of "lipid" materials (10), it appeared that not all lipids have this effect. Glyceryl monostearate in very small amounts (0.5%

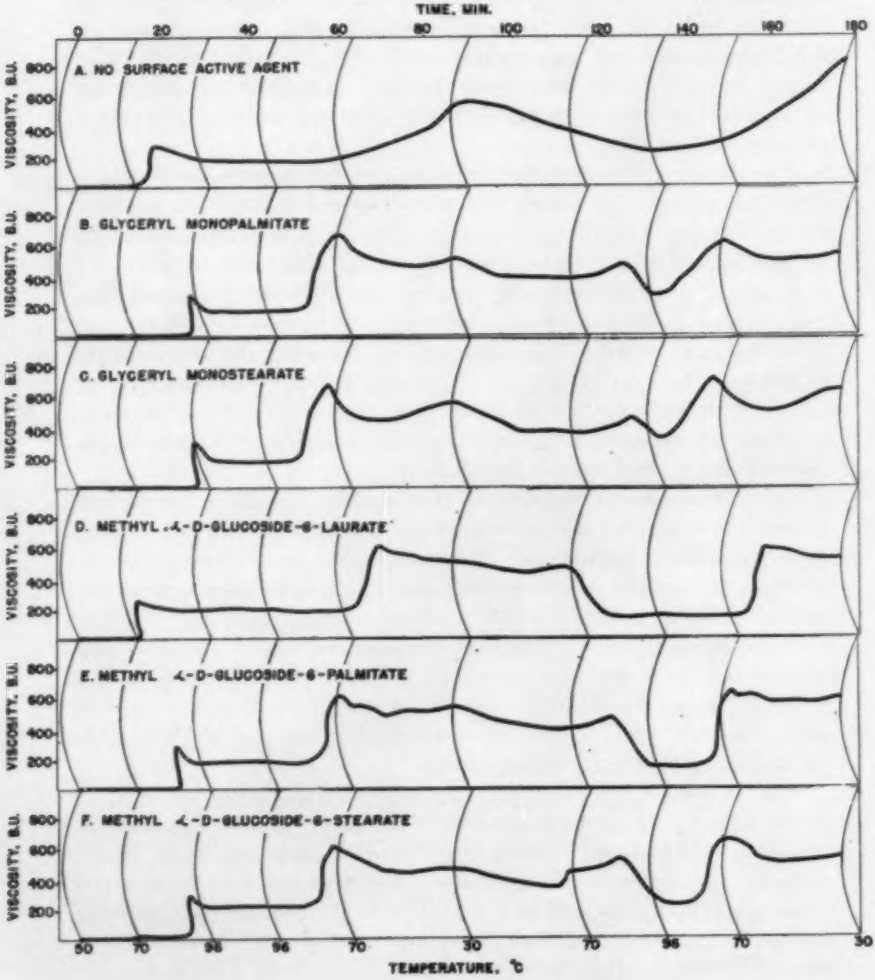

Fig. 2. Effects of 0.36% monosubstituted surface-active agents on amylograph curves of 6.5% corn starch pastes containing 5.64% soybean oil.

of defatted starch, but soybean oil and also lecithin were without any of the weight of the starch) introduced a peak into the cooling curve

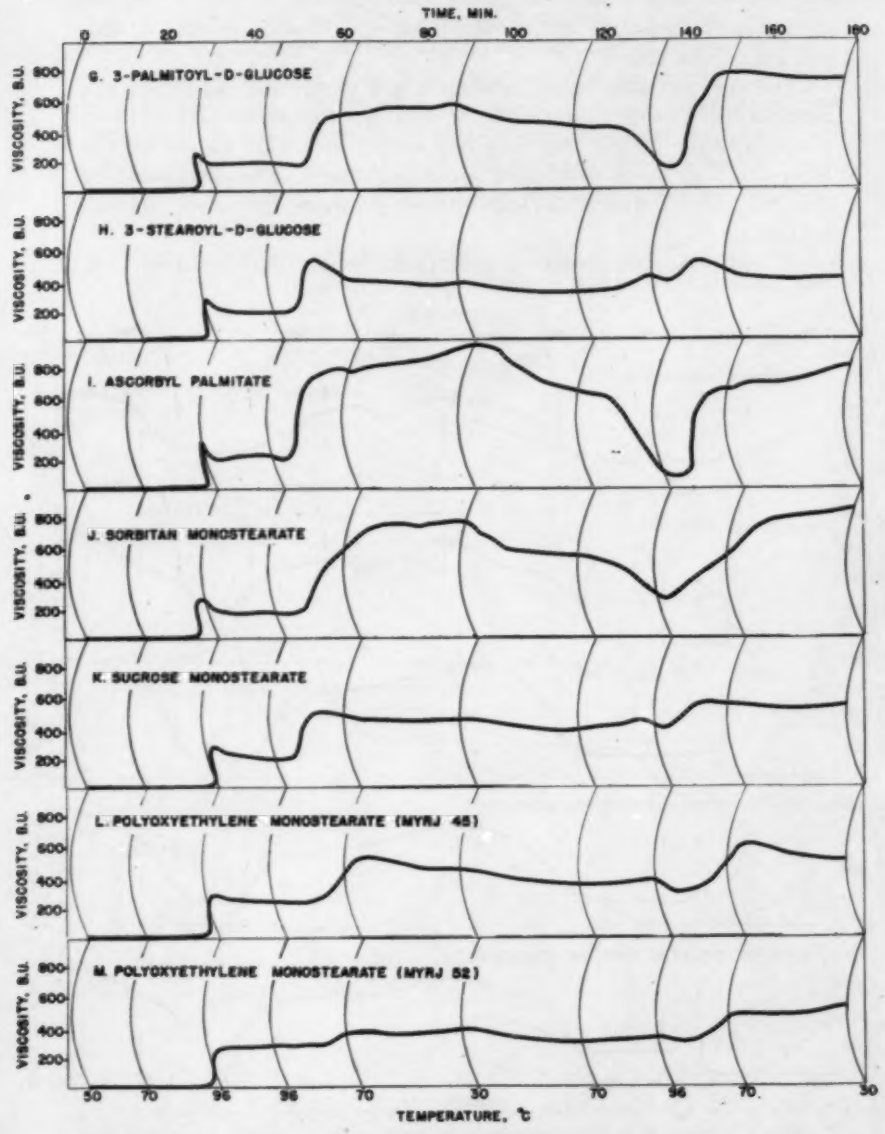

Fig. 2 (continuation).

effect. In agreement with previous observations (10), no such effects were observed with waxy corn starch. Thus it appeared that the amylose fraction and a substance with action similar to that of certain surface-active materials are needed to produce a step or peak in this portion of the curve.

The only polysubstituted substances which gave any indication of a peak in the cooling curves (Fig. 3) were heterogeneous preparations, a sucrose ester (Seqol 260) and two methyl glucoside tallow emulsifiers, which probably contained some monoesters. The corresponding diesters, sucrose distearate and methyl glucoside distearate, showed no peaks.

A few monoesters failed to show peaks in the cooling curves (Fig.

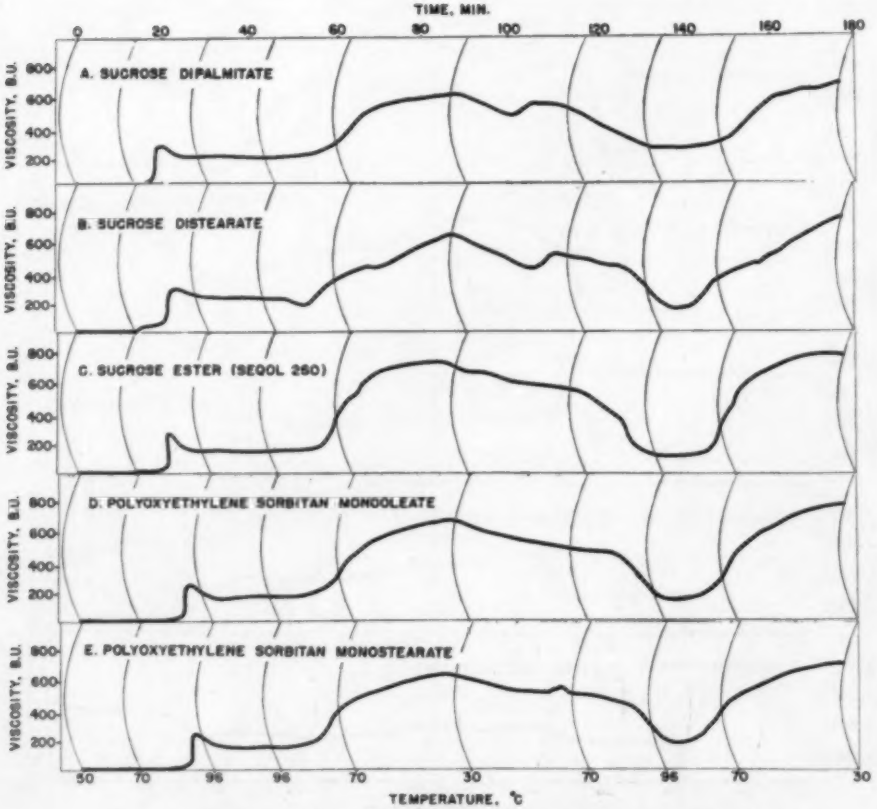

Fig. 3. Effects of 0.36% polysubstituted surface-active agents on amylograph curves of 6.5% corn starch pastes containing 5.64% soybean oil.

2). With polyoxyethylene monostearate (MYRJ 52) the fact that the compound inhibited gelatinization to such an extent that a peak was not obtained in the heating cycle was probably the cause. Ascorbyl palmitate required the addition of a large amount of sodium hydroxide to the mixture in order that the cooked paste should have the standard pH value of 6.5 used in this study. Thus the character of the compound was changed from that of the nonionic surface-active agents. In fact, the curve showed certain characteristics which were also found when sodium lauryl sulfate was substituted in the same system.

There appeared to be no obvious explanation for the lack of any definite peak in the cooling curves from 3-palmitoyl-D-glucose and

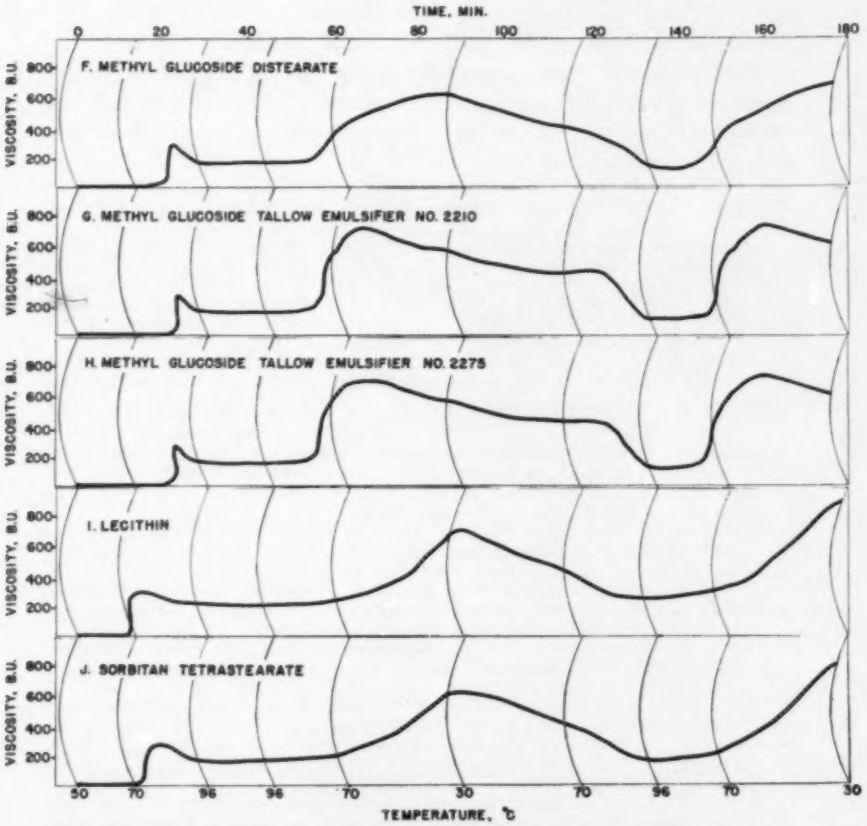

Fig. 3 (continuation).

sorbitan monostearate. However, the difference between the cooling curves of 3-palmitoyl-D-glucose and 3-stearoyl-D-glucose was somewhat comparable to the difference which has been reported for their actions as bread softeners (7,8). Although 3-stearoyl-D-glucose was judged to have excellent properties as a bread softener, 3-palmitoyl-D-glucose seemed to be almost completely lacking in such action.

All the polyesters but lecithin and sorbitan tetrastearate had pronounced effects on the shapes of the cooling curves (Fig. 3), generally causing a step to appear, but none gave rise to the peak found with most of the monoesters. Polyoxyethylene sorbitan monooleate and monostearate also produced large steps but not peaks. Although the curves with lecithin and sorbitan tetrastearate were similar to the control in shape, they were not identical with it. It thus appeared that there was some interaction between starch and all of these compounds different from that with fat alone.

Gels prepared from pastes containing any of the surface-active agents were much weaker than the control (Table II), although the effects with lecithin and sorbitan tetrastearate were much less than with the other additives tested.

Acknowledgments

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DIFFERENTIAL REACTION OF MILLED WHITE RICE VARIETIES TO A MILLON REAGENT CONTAINING TRICHLOROACETIC ACID AND MERCURIC ACETATE[1]

RUBY R. LITTLE AND GRACE B. HILDER[2]

ABSTRACT

Rice flour from 25 varieties of milled white rice showed differences in behavior of the sediment and in alteration of starch granules, as a result of treatment with a Millon reagent containing trichloroacetic acid and mercuric acetate. When the Millon treatment was followed by fluid dehydration, the sediment was either finely divided, coarsely divided, or clumped in a solid mass. Starch granules underwent slight to extreme alteration as observed in water mounts with a phase contrast microscope. The varieties studied fell into three groups with respect to coagulation of the sediment, and two groups with respect to alteration of starch granules. The two types of behavior differed in degree among varieties, and in most instances showed relationships with grain length, palatability characteristics, and other quality factors. The coagulation behavior may be useful in singling out varieties not suitable for some food-processing operations but not readily detected by presently used screening tests.

In exploring the possibilities of contributing to the basic understanding of cooking behavior in rice varieties through microscopic studies, a histochemical variation of the Millon reagent was applied to flour from samples of milled white rice. During exploratory phases of the work, when four different types of Millon reagent (4,5) were applied to flours from several rice varieties, a positive reaction for tyrosine resulted in all cases. We observed, however, that the reagent containing trichloroacetic acid and mercuric acetate produced strik-

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[2] Research staff, Human Nutrition Research Division, Agricultural Research Service, U.S. Department of Agriculture, Washington 25, D.C.

ing differences in sedimentation behavior and swelling of starch granules of different rice varieties.

The present study was undertaken to determine whether the variable response of rice varieties to this reagent might be useful in understanding other rice qualities. Differences in sedimentation behavior were determined in terms of cloudiness of the supernatant fluid, changes in sediment volume, and clumping of the precipitate during fluid dehydration. Microscopic appearance in phase contrast illumination was employed as a measure of the alteration of starch granules.

The Millon reaction is probably a complex one involving both carbohydrate and protein constituents. The observations made in this investigation are of physical and histochemical behavior, and tests were not conducted to learn the chemical reactions and interactions induced by the treatment.

Materials and Methods

The 65 lots of milled white rice investigated represented 25 varieties grown at several locations in the United States during three crop years. These were the same lots as those used for work already reported (1,6,7).

For making flour, 5 g. rice, which had been soaked in 5 ml. water at 38°C. for 1.5 hours to soften the unusually hard kernels, was ground to a paste with a porcelain mortar and pestle and spread on a watch-glass to dry. The dry material was ground and sieved repeatedly until the entire sample could be passed through a 100-mesh screen. Three or four aliquots of 0.25 g. each provided material for the replications. Aliquots from the flour samples also were used in heat-alteration evaluations (6).

Ten milliliters of Millon reagent containing 18.75% (w/v) trichloroacetic acid and 0.5 g. mercuric acetate (5) were stirred rapidly into a vial containing 0.25 g. dry rice flour. The mixture was allowed to stand 5 minutes at 38°C., after which 0.5 ml. of 1% sodium nitrite solution was added with stirring. While the mixture stood undisturbed at 38°C. in the succeeding 20-minute period, observations were made regarding the general appearance of sediment and supernatant liquid.

At the end of this 20-minute period a drop of sediment was removed from each vial with a medicine dropper and added to 5 ml. water in another vial. One drop of the dilution was placed on a slide and covered with a No. 0 coverglass; excess liquid was removed, and the cover sealed with Vaseline, for examination with a phase contrast microscope.

After another 5 minutes, liquid was decanted as well as possible

from the residue, which was then washed and dehydrated by mixing with successive increments of solvents added to the capacity of the tube (then decanting them) in the following order:

Ethanol, 70%: four times, 15 minutes' standing in the first, 5 minutes' in each subsequent treatment;
Ethanol, 95%: two times, 15 minutes' standing in the first, 5 minutes' in the second;
Absolute ethanol: two times, 20 minutes' standing in each;
Ethanol-xylene (equal parts): two times, 20 minutes' standing in each;
Xylene: two times, 20 minutes' standing in each.

During the first 70% ethanol treatment, and during the first 95% ethanol treatment, the volume and condition of the sediment were recorded. After the final treatment, the product was stored in xylene. Slides for microscopic examination were made by spreading or crushing a small amount of the sediment in a drop of Permount and adding a coverglass.

For microscopic observation, water mounts were superior to permanent preparations. The optical and photomicrographic equipment used has been described previously (6). An outline of the alteration process was developed on the basis of difference from the unaltered condition as observed in individual granules. For each replication, all starch granules within several randomly chosen microscopic fields (10×97) were assigned to one of five alteration categories. The first four, briefly characterized as unaltered, slightly altered, moderately altered, and greatly altered, were similar to those described earlier (6). The fifth category, called "extremely altered," included greatly distended granules of decreased density and ragged or indefinite outline.

Results and Discussion

Gross Observations. Behavior of treated flour from different rice varieties is shown in Fig. 1 and Table I. Rate of settling was faster, volume of the sediment less, and turbidity of the supernatant less in long-grain rices, with three exceptions, than in medium- and short-grain rices. Samples showing cloudiness in the Millon reagent also showed turbidity in the first 70% ethanol wash, and a tendency toward clumping of the sediment during dehydration. Volume of the sediment in 70% ethanol, although slightly less in long-grain than in medium-grain and short-grain rices, failed to indicate differences among varieties.

Varietal differences in sedimentation behavior were accentuated when 95% ethanol was added. In about half of the varieties, sediment volume was approximately equal to that in 70% ethanol; in the other cases sediment volume had decreased. Unchanged sediments were

either finely divided, or coarsely divided (flaky with small lumps); sediments of decreased volume were consolidated to form a single coherent mass or lump. Passage into xylene caused hardening of the lumps (Fig. 1, B) but no change in finely divided or coarsely divided precipitates.

The existence of three major groups of rice, with regard to form assumed by the sediment (finely divided, coarsely divided, or clumped), after trichloroacetic Millon treatment and dehydration, was inferred from these results.

Microscopic Observations. As observed in water mounts, few starch granules were unaltered by the Millon treatment; most had undergone slight to extreme alteration (Fig. 2). In some varieties, chiefly long-grain, most granules were slightly to moderately altered; in other varieties, most granules were greatly to extremely altered. In some varieties many granules underwent greater swelling, thinning, and

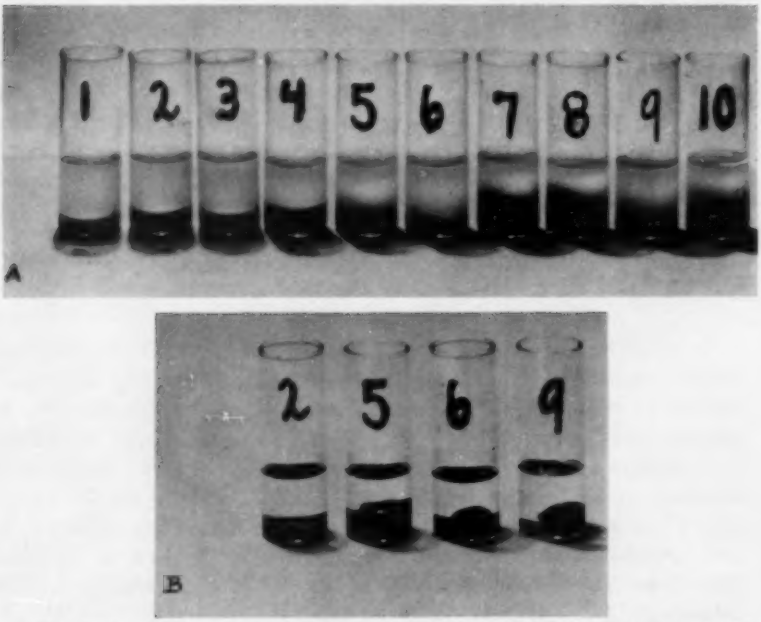

Fig. 1. Appearance of flour from representative varieties after treatment with trichloroacetic acid-Millon reagent. A, appearance after 20 minutes in the reagent; B, after dehydration and transfer to xylene. Varieties represented in A are, left to right, Bluebonnet 50, Rexoro, Texas Patna, Colusa (California), Caloro (California), Zenith, Blue Rose, Colusa (Texas), Century Patna 231, and Toro; varieties represented in B are Rexoro, Caloro (California), Zenith, and Century Patna 231.

fragmentation than is shown in the photographs.

The proportions of granules slightly to extremely altered are indicated for each variety in the last column of Table I.

On the basis of these observations, the rice varieties were divided into two major groups with regard to starch granule alteration by the trichloroacetic acid Millon reagent.

Observed in permanent slides (Fig. 3), the appearance of Millon-

TABLE I

MACROSCOPIC AND MICROSCOPIC OBSERVATIONS OF MILLED WHITE RICE FLOUR AND STARCH AFTER TREATMENT WITH TRICHLOROACETIC ACID-MILLON REAGENT

VARIETY	NO. OF LOTS	NO. OF REPLICATIONS	CLOUDINESS[a]	VOLUME OF SEDIMENT		CLUMPING[b] IN 95% ETHANOL	STARCH GRANULE ALTERATION[c]
				In 70% Ethanol	In 95% Ethanol		
				ml	*ml*		
LONG-GRAIN							
Bluebonnet	1	4	0	2.8	2.7	0	1
Bluebonnet 50	4	12	0	2.9	3.0	0	3
Century Patna 231	6	20	+++	2.5	1.8	+++*	1
Fortuna	2	8	0	3.2	3.2	0	2
Improved Bluebonnet	2	7	0	2.6	2.6	0	1
Rexark	1	4	++	2.7	1.9	+++*	4
Rexoro	4	12	0	2.6	2.6	0	1
Sunbonnet	3	9	0	2.7	2.8	0	1
Texas Patna	3	11	0	2.5	2.5	0	2
Toro	3	9	+++	3.2	2.1	+++*	5
Texas Patna 49	2	6	+	3.1	2.9	0	2
B4512A1-20	1	4	0	2.5	2.6	0	1
B4512A1-32	1	4	0	2.6	3.0	0	1
B455A1-25	2	6	0	2.6	2.8	0	1
MEDIUM-GRAIN							
Blue Rose	2	6	+++	2.9	2.1	+++*	5
Calrose (Texas)	2	6	+++	2.6	1.7	+++	5
Calrose (Calif.)	1	3	+	3.6	2.9	+	5
Early Prolific	2	6	+++	2.5	1.9	+++	1
Magnolia	2	6	+++	2.6	1.8	+++	5
Nato	2	6	+++	2.5	1.8	+++	5
Zenith	5	17	++	3.3	2.4	++	4
SHORT-GRAIN							
Calif. Pearl	1	3	++	3.3	3.0	+	5
Caloro (Texas)	4	12	++	2.9	2.1	++	4
Caloro (Calif.)	2	6	+	3.3	3.1	+	4
Colusa (Texas)	2	6	++	2.9	2.3	++	4
Colusa (Calif.)	1	3	0	3.2	3.3	+	5
11-47-11-1	2	6	+	3.4	3.4	0	5
12-47-6-2	2	6	+++	3.3	2.0	+++*	4

[a] Rating scale: 0, supernatant clear; +, ++, +++, supernatant slightly to increasingly cloudy.

[b] Rating scale: 0, sediment finely divided, not clumping; +, sediment coarsely divided, forming small lumps or large flakes or both; ++, sediment sometimes coarsely divided, sometimes coalesced to form a hard lump; +++, sediment always coalesced to form a hard lump (except starred samples, in which one or two replications were intermediate).

[c] Microscopic appearance of starch granules described as: 1, slightly and moderately altered granules predominant; 2, all categories present in about equal numbers; 3, greatly altered granules predominant but some slightly to moderately altered granules present; 4, greatly altered granules predominant and moderately altered granules infrequent; 5, greatly altered to disintegrating granules present exclusively.

affected starch was very different from that observed in water mounts. Partly gelatinized granules consisted of a luminous body, often resembling unaltered starch, surrounded by a dark rim. Wholly gelatinized granules were completely dark, or dark with a lighter center. Altered granules were reduced in size from that observed in water mounts. Materials other than starch, presumably mostly proteinaceous in nature, were observed in phase contrast. The Millon reaction color was too pale for observation with bright-field illumination at high magnifications.

Discussion. Formation of a finely divided sediment, accompanied by slight to moderate granule alteration, was generally characteristic of long-grain varieties. Partial or complete coagulation of the sediment, accompanied by great to extreme granule alteration, was generally characteristic of medium- and short-grain rices. Most of the short-grain varieties showed a more moderate clumping behavior than medium-grain varieties, and California-grown samples had this property to a lesser degree than Texas-grown samples. It was noted that in

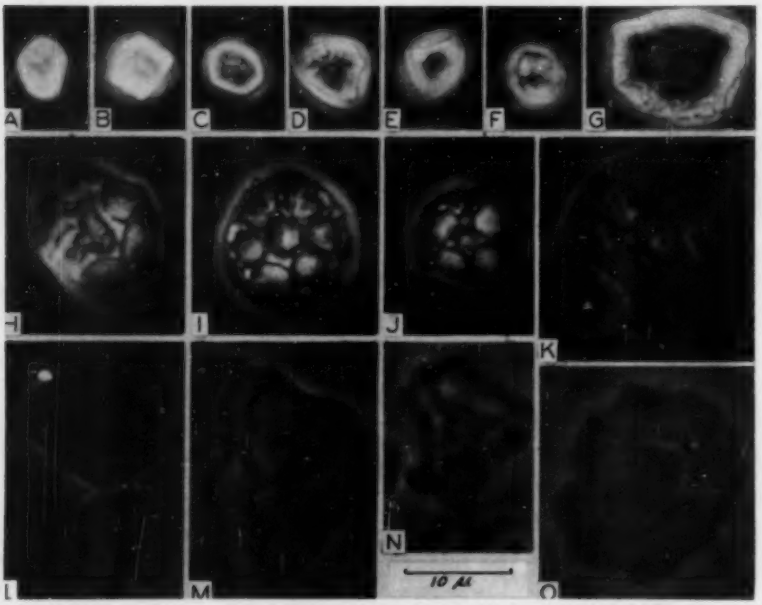

Fig. 2. Photomicrographs of starch granules in water mounts, in phase contrast illumination, illustrating alteration categories 1 (A-B), 2 (C-F), 3 (G-J), 4 (K-M), and 5 (N-O). The varieties represented are Bluebonnet 50 (E), Caloro (J), Century Patna 231 (A, F, G), Rexoro (C, H), Zenith (D, I, K, N, O), and 11-47-11-1 (L, M).

the varieties Rexark (long) and Toro (long), behavior was at variance with others of the grain length, whereas in the varieties Century Patna 231 (long), Early Prolific (medium), and 11-47-11-1 (short), starch granule alteration was at variance with clumping behavior. All of these varieties differ from others of their grain length in one or more other ways (1,2,6,7,10).

In comparing these observations with others on many of the same varieties, we see that starch granule alteration by the trichloroacetic Millon reagent is consistent with gelatinization temperature (2), reaction to dilute alkali (7), and reaction to heating in water at 62°C. (6). The clumping tendency appears to be at least partly independent of starch granule alteration; that it is seemingly consistent with low amylose content (2,10) may or may not be significant. In a side observation, rice flour prepared by comminuting in 1% salt solution and treated with Millon reagent failed to show coagulation behavior, although starch granule alteration was normal; thus a component soluble in salt solution may be a factor in sediment coagulation. The fact that trichloroacetic acid is used in histochemical procedures for cer-

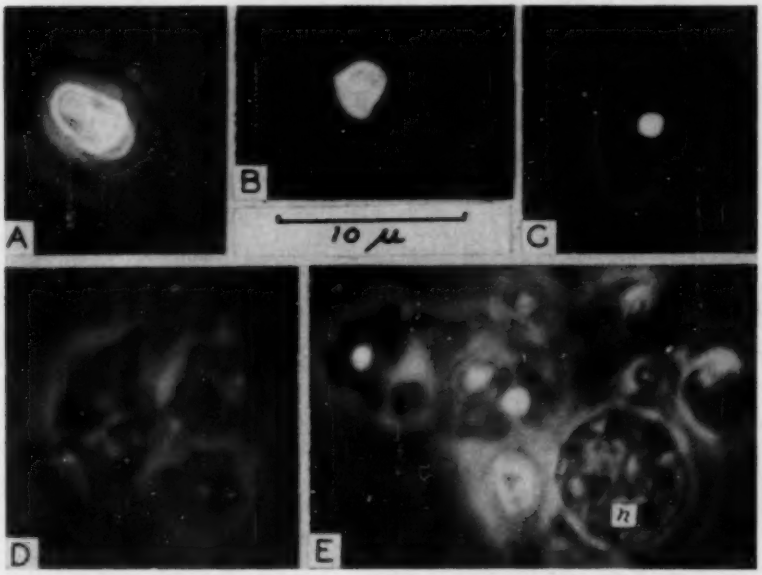

Fig. 3. Photomicrographs of Millon-treated starch granules after dehydration and mounting in Permount, illustrating degrees of alteration. The varieties represented are Rexoro (B) and 11–47–11–1 (A, C, D, E). The n (in square) indicates a body resembling a nucleus.

tain proteins or amino acids (4,9) may provide a clue.

Results indicated that the differential action of the Millon reagent containing trichloroacetic acid and mercuric acetate on starch and perhaps on other components of the rice kernel could serve as a basis on which to develop one or more tests useful in the rice industry. A sedimentation test might be developed somewhat like that of Zeleny (8,11) for wheat. As used in this investigation, the reagent separates out Century Patna 231 as the only long-grain variety showing extreme sediment coagulation along with minimum granule alteration. The reagent also seems to provide a clear-cut distinction between the Bluebonnet and Toro varieties. However, much remains to be done by way of varying methods of sample preparation, strength and composition of the reagent, conditions of treatment, and application to additional varieties and samples, before a practical test suitable for large-scale screening operations can be recommended.

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THE EFFECT OF VARIOUS SUGARS ON THE FORMATION AND CHARACTER OF GLUTEN[1]

DONNA POLAND MEISKE,[2] MARY FRANG JONES,[3] AND EVELYN M. JONES

ABSTRACT

The effects of various sugars added at 4% of flour weight were studied by baking gluten balls prepared in three ways: from gluten developed in a flour-water-sugar dough, from gluten made from a flour-water-sugar dough with the corresponding sugar again added to the gluten after washing, and from gluten made from a flour-water dough with sugars added to the gluten after washing.

In most instances addition of sugars to gluten resulted in no significant changes in volume of baked gluten balls. Tenderness of baked gluten balls was increased significantly over controls only when sugar was again added to gluten developed in the presence of sugar. Sugar added to raw gluten had a dehydrating effect on the raw gluten as shown by extent of drip loss.

In addition, several sugars were added in increasing amounts with constant flour and water to form doughs until no gluten was recovered when the dough was washed. At concentrations varying from 25 to 45% of flour weight, all of the sugars used except the relatively insoluble alpha-lactose interfered severely with gluten formation. Tenderness of baked gluten from these treatments seemed to increase at a greater rate than could be accounted for by decrease in yield alone.

It is commonly accepted that sucrose tenderizes baked products. Most food textbooks which offer an explanation attribute this effect of sugar to a peptizing action on flour proteins, but give no supporting references. Work of Jago and Jago (3,4) in 1911, and again in 1921, showed that the physical condition of a flour-water dough was noticeably affected by the presence of sucrose. As the concentration of the sugar in the dough increased, dough viscosity decreased. They also found that the amount of gluten recovered from a dough made with sucrose, flour, and water was less than that recovered from a flour-water dough. They proposed that the sugar diminished the water-absorptive power of the flour proteins and that it also exerted a solvent action on the flour proteins. In 1958, Baxter and Hester (1) proposed that sucrose interfered with gluten development owing to competition between gluten and the sugar for water, since when sucrose was added after gluten was developed, about the same amount of wet gluten was recovered from the dough as in the control, but the amount recovered was reduced when sucrose was added before gluten

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[2] Present address: St. Paul, Minnesota.

[3] Present address: Ames, Iowa.

was developed.

The experiments reported in this paper include studies on the effects of various sugars on gluten formation and character and on the volume and tenderness of baked gluten balls.

Materials and Methods

A commercial, all-purpose, bleached flour containing 12.4% protein (14% moisture basis) was used. The sugars included were D-fructose (C.P.), D-glucose (C.P.), maltose (Tech.), beta-lactose (Tech.), and sucrose. Mixing was done in a Kitchen-Aid mixer, Model 3C.

For Part A, Section I, the gluten was prepared by mixing 1,000 g. flour with 800 g. water, washing by hand until the washwater gave a negative starch-iodine test, and draining. The gluten was divided into 60-g. lots. To a lot of gluten, 7.2 g. of a sugar were added and blended for 10 minutes in the mixer. The amount of sugar was equivalent to 4% of the weight of flour used in the dough. Two lots of gluten were treated in the same way, but with no sugar, to serve as controls. After weighing, each lot of gluten was divided into four equal parts, shaped into balls, baked for 15 minutes at 232°C., and for an additional 35 minutes at 149°C. (5).

For Part B, Section I, the gluten was prepared by mixing 355 g. flour, 288 ml. water, and 7.2 g. of sugar and washing as in Part A. The amount of flour was that estimated from Part A to give 120 g. gluten. The total yield of gluten was weighed, divided into two equal parts of approximately 60 g. each, and to one part (Part B-1) 7.2 g. of the corresponding sugar was added as before on the assumption that it had been lost during washing of the gluten. Gluten for controls for this part of the study was made in the same way, but with no sugar present at any time. Again, each 60-g. lot of gluten was divided into four equal parts, shaped into balls, and baked.

The difference to the nearest 0.1 g. between the weight of prepared wet gluten (or the gluten and added sugar) and its weight after 10 minutes of mixing was designated as drip loss. Volumes, by the rapeseed method, and tenderness of baked gluten balls were measured 1 hour after removal from the oven. A crude method was devised for estimating tenderness by measuring the weight required to crush the gluten ball, as follows: A gluten ball was placed inside a 1-liter glass beaker. A hollow cylinder which fitted exactly inside the beaker was placed on top of the ball. Metal shot was released from a funnel hanging above the cylinder in the beaker, at a uniform rate until the gluten ball was completely crushed. The cylinder and shot were then weighed. In this way the amount of weight needed to crush a gluten

ball was measured and the relative tenderness was determined. Since tenderness was expressed as g. required to crush a gluten ball, a lower number indicates a higher relative tenderness.

Section II of the experiment was concerned with the effect of varying amounts of different sugars, expressed as percent of flour weight, on yield of gluten and on volume and tenderness of baked gluten balls. Five percent increments of a sugar were added to form a dough with 30 g. of flour and 24 ml. of water until no gluten was recovered. In addition to the sugars used in Section I, maltose (C.P.) and alpha-lactose (C.P.) were included. The dough was mixed, rested and washed four times, 2 minutes each, in 750 ml. tap water, using the mixer. When the washwater was passed through a wire sieve (20-mesh), the gluten was held in the sieve while starch, solubles, and other finely dispersed material passed through. If the total mixture passed through the sieve, it was assumed that no gluten was formed.

After the washing was completed, the gluten was drained and mixed again in the mixer for 10 minutes. It was then weighed, shaped into a ball, and baked. The baking procedure was the same as that used in Section I. When gluten yields of less than 3 g. were obtained, the mixing times and baking times were shortened.

Volumes and tenderness were determined as in Section I.

Results and Discussion

Section I

The mean volumes and tenderness of baked gluten balls and mean drip losses of raw gluten are shown in Table I. Studentized range (2) was used to test statistical differences among averages when analysis of variance revealed that there were significant differences.

When considered as a group, volumes of baked balls prepared from gluten obtained from a dough containing sugar, 552 ml. (Part B-1), and 594 ml. (Part B-2), were somewhat greater than those made from gluten containing no sugar, 473 ml. (Part A). Gluten for Part B was made in smaller quantities than that for Part A and the control balls from Part A had smaller volumes than control balls from Part B. Therefore these differences between groups were not considered great enough to be significant.

Individual analysis of volumes of gluten balls within Part A (gluten prepared in the absence of sugar) showed that only the gluten balls to which beta-lactose or maltose had been added had significantly smaller volumes than control balls. The maltose was later found to be very impure which probably influenced this result. Lactose should have had a similar effect in Part B-1, but statistical analysis showed that volumes

TABLE 1

INFLUENCE OF 4% LEVELS OF SUGARS AND METHODS OF INCORPORATING SUGARS ON THE DRIP LOSSES OF GLUTEN, AND THE VOLUMES AND TENDERNESS OF BAKED GLUTEN BALLS

Sugars Added to Raw Gluten and/or Used in Dough Preparation	Method of Preparation of Gluten								
	Part A: Flour-Water Dough (Sugar Added to Prepared Gluten)			Part B-1: Flour-Water-Sugar Dough (Additional Sugar Mixed with Prepared Gluten)			Part B-2: Flour-Water-Sugar Dough (No Additional Sugar Added to Prepared Gluten)		
	Drip Loss[a]	Volume[b]	Tenderness[c]	Drip Loss	Volume	Tenderness	Drip Loss	Volume	Tenderness
	g	*ml*	*g*	*g*	*ml*	*g*	*g*	*ml*	*g*
Sucrose	14.0	501	2478	13.4	563	2139	3.8	625	3180
D-fructose	13.7	546	2197	13.9	554	2061	4.5	573	2998
Maltose	12.9	434	2647	11.1	591	2104	5.1	598	3161
Beta-lactose	14.7	420	2633	14.4	518	2520	3.6	580	2870
D-glucose	13.7	464	2558	13.1	533	2142	3.7	595	3285
Group Mean	13.8	473	2503	13.2	552	2193	4.1	594	3099
Control (no sugar)	3.8	546	2602	3.0	623	3352	3.0	623	3352

[a] Mean drip loss of four 60-g. lots of gluten.
[b] Mean volume of the 16 gluten balls obtained from four 60-g. lots of gluten.
[c] Mean weight in g. needed to crush one gluten ball. Smaller number indicates greater tenderness.

for gluten prepared from the dough containing this sugar were not significantly different from those from doughs containing other sugars.

The weight needed to crush gluten balls containing no sugar (3,352 g.) was decreased significantly by treating with a sugar only in Part B-1 (2,193 g.) when the sugar was again added to gluten prepared in the presence of the sugar. Smaller weight was needed to crush gluten balls in Part A (2,503 g.) and Part B-1 (2,193 g.) where sugar was added to prepared gluten than in Part B-2 (3,099 g.) where sugar was present during dough formation only.

Drip losses, which included sugar as well as water and other water-soluble materials, were greater when the sugars were incorporated after preparation of gluten (13.8 g., Part A, and 13.2 g., Part B-1, as compared with 4.1 g., Part B-2); it therefore appears that the presence of sugars decreases the hydration of gluten.

Section II

When sugars were included in increasing amounts (at 5% increments) during dough formation, there was no great change in gluten yield for each sugar at the lower concentrations (Table II). However, a "critical level" was found for each sugar except alpha-lactose where gluten yield dropped off sharply as follows: fructose, glucose, and sucrose, 55–65%; maltose, 45%; and beta-lactose, 35–45%. The alpha-lactose did not affect gluten yield, even at 70%, the highest concentration used.

Data on gluten ball volumes are not presented, since volume

TABLE II

PERCENT CHANGE IN YIELD OF GLUTEN AND TENDERNESS OF GLUTEN BALLS WITH VARIATION IN KIND AND CONCENTRATION OF SUGAR[a]

Sugar	Decrease in Yield (Y) and Increase in Tenderness (T)											
	Fructose		Glucose		Sucrose		Maltose		Beta-Lactose		Alpha-Lactose	
	Y[b]	T[b]	Y	T	Y	T	Y	T	Y	T	Y	T
%	%	%	%	%	%	%	%	%	%	%	%	%
0	0	0	0	0	0	0	0	0	0	0	0	0
5	9	16	9	16	0	35	0	10	8	14	9	5
15	0	15	0	23	0	26	0	40	17	50	0	29
25	9	23	18	41	0	29	10	56	50	50	9	27
35	18	50	36	62	9	43	60	75	100	..	9	21
45	64	79	82	69	54	70	100	..	100	..	9	13
Control (g.)	11	4165	11	3740	11	4770	10	4333	12	4118	11	3894

[a] Actual figures are given at the bottom of the table for control in g. yield or g. required to crush gluten balls. Each figure was an average of three replications.
[b] Y = yield; T = tenderness.

changes were slight until gluten yields were greatly reduced. Then volumes decreased as expected. In the alpha-lactose series volumes were never reduced significantly. Tenderness increased with increases in sugar. For all sugars except alpha-lactose, a 40 to 50% decrease in weight needed to crush the gluten balls was achieved with a maximum of 18% decrease in gluten yield. Thus when one considers the percent change in tenderness and gluten yield it appears that tenderness increased to a greater extent than could be explained by a decrease in gluten yield alone.

These results indicate that a sugar must be in solution to exert an effect on gluten formation. The alpha-lactose, which is less soluble than the other sugars, exerted no significant effects on gluten yields or volumes of baked gluten balls. Tenderness changes also seem too small to be significant.

It is suggested that all of the sugars except alpha-lactose exerted a solvent or peptizing action on the formed gluten and/or interfered with water absorption by the gluten proteins and thus interfered with gluten formation. Hence, as increasing percentages of sugar were added, less gluten was recovered, and at "critical concentrations" no gluten was recovered.

Acknowledgments

The authors are indebted to William Baten, Statistician, Agricultural Experiment Station, East Lansing, for assistance with the statistical analyses; and to Flora Hanning, Foods and Nutrition Department, School of Home Economics, University of Wisconsin, Madison, for helpful discussions.

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RELATION OF VIABILITY AND STORAGE DETERIORATION TO GLUTAMIC ACID DECARBOXYLASE IN WHEAT[1]

PEKKA LINKO AND LARS SOGN[2]

ABSTRACT

Glutamic acid decarboxylase activity of 25 commercial wheat samples was highly correlated with percentage of germ-damaged or "sick" wheat ($r = -0.878^{***}$), germination percentage ($r = +0.921^{***}$), topographical tetrazolium test ($r = +0.912^{***}$), fat acidity ($r = -0.864^{***}$), and fluorescence ($r = -0.637^{***}$). The correlation involving germ damage is significantly higher (at 5% level) than that involving fluorescence. With 19 samples of new crop wheats of little germ damage and high germination percentage, the correlation between glutamic acid decarboxylase activity and viability was insignificant ($r = +0.185^{ns}$), largely owing to differences in decarboxylase activity of wheats from various locations and of different variety. It was concluded that though glutamic acid decarboxylase activity seemed to have little value in examining new crop wheats of high viability, either alone or together with other tests it may give a good picture of the storage background of wheat.

Wetting of wheat embryos causes a rapid decrease in the amount of free glutamic acid with simultaneous carbon dioxide evolution, and an increase in free gamma-aminobutyric acid (28,29). Though this was due to the immediate activation of glutamic acid decarboxylase in moistened wheat (7,27), glutamic acid decarboxylase activity decreases during prolonged storage, particularly at elevated moisture levels (7,37). Preliminary evidence indicated a possible correlation between decarboxylation of glutamate and viability, or storage deterioration, respectively.

Several enzyme reactions have been previously related with the viability of seeds. In 1922 Turesson (41) introduced a method using the enzymatic reduction of methylene blue as an indicator of viability. A few years later Russian workers used successfully indigo carmine for testing quick germinating seeds (cf. 36). Eidmann (14), Gadd and Kjaer (17), and Lakon (22) employed the reduction of selenium salts as germination indicator. Later Lakon (23,24) studied the enzymatic reduction of several tetrazolium derivatives in this respect, finding 2,3,5-triphenyl tetrazolium chloride the most suitable. Sorger-Domenigg *et al.* (40) developed a method for colorimetric estimation

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[2] Respectively, Assistant Professor and W. K. Kellogg Foundation Fellow, Kansas State University, Manhattan.

of the reduction product formazan. Amylase (16,32), catalase (12,13, 25), and peroxidase (5) activities have also been correlated with storage deterioration and viability.

In the present study various factors involved in the deterioration of wheat grain have been investigated, with special attention to biochemical methods in determining the degree of viability. The suitability of various methods in estimating storage deterioration of wheats of different background was studied, with emphasis on the relation of germ damage to the application of glutamic acid decarboxylase activity as an index of deterioration and viability.

Materials and Methods

A total of 49 wheat samples was investigated. Twenty-five of the samples were commercial mixtures of hard red winter wheats at various stages of deterioration (series 1). These samples had been stored at 4°C. for about a year before the analyses were made. Twenty samples were new crop wheats (eight hard red spring and twelve hard red winter) of relatively high viability (series 2), and four were frost-damaged wheats (series 3). Moisture content varied from 9.4 to 12.9%. All quantities used for various analyses, as well as results, are reported on a moisture-free basis. Unless otherwise mentioned, all experiments were performed in duplicate.

When whole grains were used for various determinations, they were ground in a Waring Blendor for 2 minutes. For experiments with wheat embryos, the germ ends of kernels were carefully cut off with a razor blade so that no germ remained in the endosperm end. The isolated germ ends were ground in a mortar.

Germ-Damaged or "Sick" Wheat. Germ damage was determined from 200-kernel samples by carefully removing the pericarp covering the embryo. Cream-colored or white embryos were considered alive and the wheat was classified sound. In all other cases wheat was judged as damaged ("sick").

Germination Test. One-hundred-kernel samples were surface-sterilized by soaking 2 minutes in 0.1% mercuric chloride solution, followed by a thorough rinsing in tapwater. The kernels were placed crease down on moist, sterile quartz sand in Petri dishes, covered with wet filter paper, and allowed to germinate in the dark at 24° to 26°C. for 7 days. Grains with normal sprouts were counted and removed every second day. Results were confirmed by the Seed Laboratory, Kansas State Board of Agriculture, Topeka.

Fluorescence. Fluorescence was determined according to a slightly modified procedure of Cole and Milner (10). One gram of ground

kernels or 250 mg. of ground germ ends were weighed into Erlenmeyer flasks each containing 25 ml. of 0.2*M* hydrochloric acid. The mixtures were shaken by hand at certain time intervals, and allowed to stand overnight at 25°C. After filtering through Whatman No. 5 paper, the clear solutions were diluted, if necessary, and used for fluorescence determinations. Measurements were made with a Coleman Photoelectric fluorometer with B_1-S and PC-1 filters; the instrument was standardized to read 60 with 0.1 p.p.m. sodium fluorescein solution.

Fat Acidity. Fat acidity determination was based on the procedure described in *Cereal Laboratory Methods* (1). Ten grams of freshly ground material were extracted in a Goldfisch extractor with 50 ml. of petroleum ether (Skelly Solve B; b.p. 63° to 69°C.) for 6 hours. After removal of the solvent, the extract was dissolved in 25 ml. of isopropyl alcohol-benzene-water mixture (48:50:2, by volume) containing 0.2% of phenolphthalein, and titrated to a distinct pink color with 0.0129*N* potassium hydroxide solution. Fat acidity was reported as mg. of potassium hydroxide required to neutralize the free fatty acids in 100 g. of ground material.

Dehydrogenases. Dehydrogenase activity was determined by two different methods, both based on the enzymatic reduction of 2,3,5-triphenyl tetrazolium chloride (TTC) to formazan. The first method was a modification of Lakon's (23) topographical test. The kernels were soaked overnight in distilled water at 4°C., cut lengthwise, and again soaked 12 hours in darkness in 1% TTC-solution (M/15 phosphate buffer, pH 7.3) at 25°C. The percentage of colored embryos in 200 kernels was then estimated by visual examination. Only kernels with completely stained embryos and scutellums were judged viable.

The second method was a modification of that proposed by Sorger-Domenigg *et al.* (40). Five milliliters of 1% TTC-solution were added to a test tube containing 1 g. of ground kernels or 250 mg. of ground germ ends. After two vacuum infiltrations the test tubes were incubated 1 hour at 38°C. The formazan produced was extracted with 25 ml. of acetone, and the extinction was measured at 520 $m\mu$ with a Beckman Model DU spectrophotometer.

Decarboxylases. Glutamic and pyruvic acid decarboxylase activities were determined manometrically as previously described by Cheng *et al.* (8). The activities were reported as microliters of carbon dioxide produced by 500 mg. of ground kernels or 100 mg. of ground germ ends during 30 minutes from 1 ml. of 0.1*M* substrate solution (M/15 phosphate buffer, pH 5.8). Determinations were performed in triplicate. The standard deviation of decarboxylase activity determinations was $\pm 2\%$. The reproducibility was also determined by calculating

intraclass correlation coefficients of all triplicate runs in a series according to Fischer (15). With whole kernels $r_I = +0.984^{***}$, and with germ ends $r_I = +0.978^{***}$ were obtained.

Results and Discussion

Viability and Germ Damage. Table I shows that series 1 covered a relatively wide germination range, whereas in series 2, with one exception (sample No. 20, germination 0%) the germination percentages were high, falling within the range of 77 to 97%. The high viability in series 2 is understandable for new crop wheats. The germination of frost-damaged wheats varied from 74 to 96%.

As shown by Tables II and III, a high negative correlation was found between the quantity of germ damage and germination percentage ($r = 0.969^{***}$ in series 1; $r = -0.867^{***}$ in series 2). These values agree well with the results of Cole and Milner (10), who also obtained a high correlation ($r = -0.828^{***}$; calculated from their values). On the other hand, Sorger-Domenigg *et al.* (40) reported a relatively low correlation between germ damage and viability ($r = -0.491^{***}$), evidently due to difficulties in estimating germ damage. Since the loss of viability appears to precede visually detectable browning of the germ, it is necessary to classify kernels with even slightly browned embryos damaged. In series 1, correlations between germ damage and germination percentage or topographical TTC-test, respectively, were significantly higher (at 5% level) than those involving other measurements. On the other hand, the correlation between germ damage and glutamic acid decarboxylase activity was significantly higher (at 5% level) than that involving fluorescence.

Fat Acidity. It has been known for some time that germ damage in wheat is followed by high fat acidity (6,34,35,42). As shown by Table I, fat acidity values in series 1 varied from 19.5 to 45.2, and in series 2 from 9.1 to 13.2. According to Tables II and III, fat acidity has a high negative correlation with germination percentage ($r = -0.906^{***}$ in series 1; $r = -0.920^{***}$ in series 2) and with glutamic acid decarboxylase activity ($r = -0.864^{***}$ with whole kernels, series 1; $r = -0.696^{***}$ with whole kernels, series 2). However, there seems to be no correlation between fat acidity and viability with new crop wheats of high viability (Table II). In both series 1 and 2 the correlation between fat acidity and germ damage was not significantly different (at 5% level) from that involving germination percentage. Sorger-Domenigg *et al.* (40) obtained a correlation $r = -0.915^{***}$ between fat acidity and viability, but they found a very low correla-

TABLE I

DISTRIBUTION OF VARIOUS CHARACTERISTICS OF WHEAT SAMPLES
(Roman type = minimum; italic type = maximum value)

SERIES	NUMBER OF SAMPLES	GERMINATION	GERM DAMAGE	FAT ACIDITY	FLUORESCENCE	GA DECARB. ACTIVITY	TOPOGR. TTC-TEST	COLOR. TTC-TEST
		%	%	*mg*	*E/g*	*µl*	*E/g*	*E/g*
1	25	0	19	19.5	20	4.4	0	0.03
		90	*100*	*45.2*	110	*95.8*	*89*	*0.43*
2	19[a]	77	1	9.1	11	64.7	90	
		97	*23*	*13.2*	*50*	*111.6*	*97*	
3	4	74	9	14.8	39	93.7	86	
		96	*32*	*21.4*	*86*	*106.0*	*95*	

[a] One sample (No. 20) which showed 0% germination and 60% germ damage was not included in these data.

TABLE II

SIMPLE CORRELATION COEFFICIENTS IN SERIES 1
(Values obtained with whole kernels in roman type, those with germ ends in italics; all coefficients are significant at 0.1% level)

	GERMINATION	GLUTAMIC ACID DECARBOXYLASE ACTIVITY	FLUORESCENCE	TOPOGRAPHICAL TTC-TEST	COLORIMETRIC TTC-TEST	FAT ACIDITY
	%					
Percent germ damage	−0.969	−0.878	+0.804	−0.973	−0.859	+0.837
		−0.882	*+0.858*		*−0.690*	
Percent germination		+0.921	−0.758	+0.990	+0.902	−0.906
		+0.933	*−0.791*		*+0.766*	
Glutamic acid decarboxylase activity			−0.637	+0.912	+0.873	−0.864
Fluorescence						+0.590
Topographical TTC-test					+0.902	

TABLE III

SIMPLE CORRELATION COEFFICIENTS WITH WHEATS FROM 1959 CROP (SERIES 2)

CORRELATION	CORRELATION COEFFICIENTS	
	All Samples	Without Sample No. 20
Germination and germ damage	−0.867***	−0.195[ns]
Glutamic acid decarboxylase activity and germ damage	−0.682***	
Glutamic acid decarboxylase activity and germination	+0.744***	+0.185[ns]
Glutamic acid decarboxylase activity and fat acidity	−0.696***	
Topographical TTC-test and germination	+0.971***	+0.849***
Fluorescence and germination	−0.957***	−0.412**
Fat acidity and germination	−0.920***	+0.042[ns]
Fat acidity and germ damage	+0.825***	
Topographical TTC-test and germ damage	−0.876***	

tion between fat acidity and germ damage percentage ($r = +0.457^{***}$). On the other hand, the correlation coefficients $r = +0.81^{***}$ (2) and $r = +0.847^{***}$ (3) obtained by Baker and co-workers between fat acidity and germ damage are virtually the same as found in this study ($r = +0.837^{***}$ in series 1; $r = +0.825^{***}$ in series 2).

Zeleny and Coleman (42) emphasized the importance of three types of free acidic compounds in cereal grains in relation to the degree of deterioration, namely, fatty acids, acid phosphates, and amino acids. According to them phosphates and amino acids increase significantly only in wheat samples that have undergone a considerable degree of deterioration, whereas a highly significant increase in fat acidity may appear at very early stages of deterioration. Linko and Milner (29) have shown, however, that rapid changes in the composition of free amino acids may occur. Glass and Geddes (18) reported recently that deteriorating wheat exhibits an increase in inorganic phosphorus, apparently due to the action of phytase on phytic acid. Although they observed a greater increase in fat acidity than in inorganic phosphorus, the latter increased more rapidly at the early stages of deterioration. The results from the present study as compared with those previously obtained clearly suggest that increased fat acidity is an indication of lowered viability, though it does not have to be primarily associated with any of the known deteriorative processes involved.

Fluorescence. Previous work in this laboratory (10,33) has shown that extracts of damaged wheat embryos exhibit an increase in fluorescence, which precedes the respiratory increase indicative of mold growth. On the other hand, fluorescence of sound wheat samples of widely different source, variety, and class has been found low and virtually uniform. In the present study the fluorescence value was determined both with whole kernels and with isolated germ ends. The correlation between these two measurements is very high ($r = +0.920^{***}$). There was generally little change in fluorescence in samples of 20 to 100% germination (Fig. 1). The correlation between fluorescence and germination percentage in all of the 25 samples of series 1 was $r = -0.758^{***}$. This value agrees well with results obtained by Cole and Milner (10; $r = -0.775^{***}$), and Sorger-Domenigg *et al.* (40; $r = -0.663^{***}$). However, when correlation coefficients were calculated, neglecting four extreme samples of very high percentage of germ damage, a poor correlation was obtained ($r = -0.479^{**}$). Similar results were obtained with series 2, as shown by Table III. In this case the over-all correlation between fluorescence and germination percentage ($r = -0.957^{***}$) was significantly higher

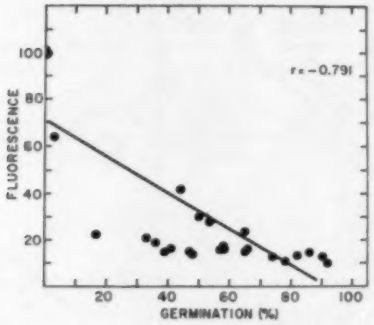

Fig. 1. Relation between fluorescence and germination percentage in series 1.

(at 0.1% level) than the same correlation after neglecting the extreme sample No. 20 ($r = -0.412^{**}$), indicating that sample No. 20 greatly influences the correlation. Figure 1 suggests that at very early stages of deterioration little increase in fluorescence appears to take place. With advancing deterioration fluorescence increases, accompanied by an increase in distribution of values obtained with wheat samples of the same viability. In particular, unexpectedly high fluorescence values may be encountered in samples of little or no viability, supporting the theory (26) that primary browning products formed during the early stages of deterioration are not fluorescent; viability may be lost before any browning of the embryo can be visually detected, and browning in turn precedes any marked increase in fluorescence. After viability is lost, nonenzymatic browning advances, leading to highly fluorescent compounds.

Glutamic Acid Decarboxylase Activity. Although Cheng *et al.* (9) showed that the marked immediate carbon dioxide evolution from wetted wheat germ is due to enzymatic decarboxylation of free glutamic acid, evidence for a negative correlation between glutamic acid decarboxylase activity and germ damage or fluorescence, respectively, was obtained during storage of moist wheat (7). Király and Farkas (21) found a markedly decreased glutamic acid decarboxylase activity in wheat infected by stem rust *(Puccinia graminis).* As can be seen from Table I and Fig. 2, different samples of wheat in series 1 exhibited a relatively large variation in the decarboxylation of glutamate. Glutamic acid decarboxylase activity in series 1 was highly correlated with germ damage ($r = -0.878^{***}$), viability ($r = +0.921^{***}$), topographical TTC-test ($r = +0.912^{***}$), fat acidity ($r = -0.864^{***}$), and fluorescence ($r = -0.637^{***}$). Though the simple linear correlations between germination percentage and glutamic acid decarboxy-

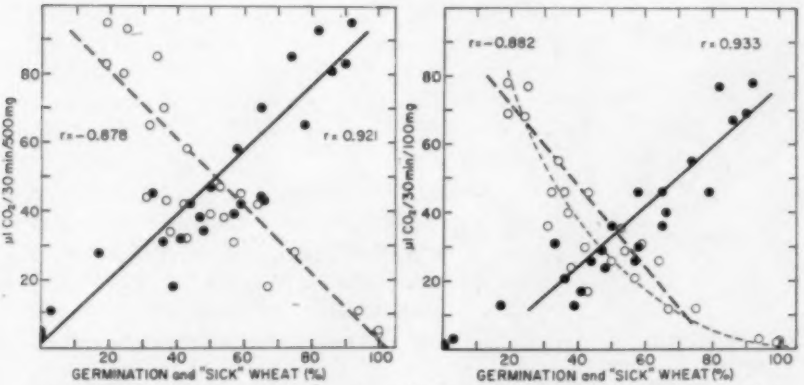

Fig. 2. Relation between glutamic acid decarboxylase activity and germination percentage (black circles), or "sick" wheat (germ damage) percentage (white circles) in series 1. Left: determinations with whole kernels; right: determinations with isolated germ ends.

lase activity are high and significant (Table II), it appeared likely that the enzyme activity decreases relatively faster at the early stages of deterioration, when only a few kernels have totally lost their viability.

In series 2 the correlation between glutamic acid decarboxylase activity and viability ($r = +0.744^{***}$) was significantly lower (at 5% level) than in series 1. Again, the neglect of sample No. 20 in statistical calculations resulted in an insignificant correlation (Table III). Wheats in series 2 were obtained from widely different sources, differing as to variety and growth conditions. All were from the 1959 crop. It has been shown previously that marked differences in the activity of glutamic acid decarboxylase occur in different varieties of wheat, as well as in wheats from various locations. It is, therefore, most likely that the varietal differences in new crop wheats of high viability are about the same order of magnitude as differences due to the beginning deteriorative processes. This led to the conclusion that though glutamic acid decarboxylase activity seemed to have little value in examining new crop wheats, it alone or together with other tests may give a good picture of the storage background of wheat.

Carboxylase Activity. A preliminary study (7) had indicated that there were virtually no varietal differences in regard to pyruvic acid decarboxylase activity in wheat grains. As can be seen from Table IV, the correlation coefficient in series 1 between germ damage and carboxylase activity is significantly higher (at 5% level) than that involving glutamic acid decarboxylase activity, but no significant differ-

TABLE IV

COMPARISON OF PYRUVIC AND GLUTAMIC ACID DECARBOXYLASE ACTIVITIES IN RELATION TO GERM DAMAGE AND VIABILITY. CORRELATION COEFFICIENTS

	Series 1		Series 2	
	Pyruvate	Glutamate	Pyruvate	Glutamate
Percent germ damage	−0.940**	−0.759*		
Percent germination	+0.973**	+0.985***	+0.494ns	−0.088ns

ence was found in regard to viability. The five samples examined for their carboxylase activity in series 2 were chosen so that there was no correlation between glutamic acid decarboxylase activity and germination percentage. The correlation involving carboxylase was numerically greater, but still insignificant, evidently owing to the small number of samples investigated.

Tetrazolium Tests. Though the topographical TTC-test has been widely accepted as a means of detecting viability of seeds (11,20,36, 38,39), difficulties in evaluation of the results have been also reported (4,19,30,31). In the present study the topographical TTC-test generally gave the best correlation with both germination and germ damage percentages (Tables II, III, and V). However, the differences between correlations involving TTC-test and fat acidity, respectively, in series 2 were not significantly different at the 5% level. The correlations between TTC-test and viability were significantly higher (at 0.1% level) than correlations involving glutamic acid decarboxylase activity and viability. Even when sample No. 20 in series 2 was neglected in statistical calculations (Table III), a high positive correlation between TTC-test and germination percentage was obtained ($r = +0.849^{***}$). The correlation between the topographical TTC-test and germination percentage ($r = +0.990^{***}$) in series 1 was significantly higher (at 0.1% level) than that involving the colorimetric TTC-test ($r = +0.902^{***}$). This agrees with the relatively low correlation obtained by Sorger-Domenigg *et al.* (40; $r = +0.602^{***}$). It was interesting that a high positive correlation exists between the topographical TTC-test and glutamic acid decarboxylase activity in series 1 ($r = +0.912^{***}$ with whole kernels).

Frost Damage. Germination percentage was found to decrease and

TABLE V

SIMPLE CORRELATIONS WITH FROST-DAMAGED WHEAT SAMPLES

	Percent Germ Damage	Glutamic Acid Decarboxylase Activity	Fluorescence	Topographical TTC-Test	Fat Acidity
Percent germination	−0.982**	−0.608ns	−0.926*	+0.996***	−0.933*

germ damage, fat acidity, and fluorescence to increase with increasing relative amount of frost damage (Table V). This was contrary to the observations of Baker *et al.* (3), who found low fat acidity values regardless of the degree and amount of frost damage. They suggested that freezing of wheat does not cause hydrolytic deterioration of fats. However, it seems quite likely that during prolonged storage deteriorative processes may be even faster in grains initially affected by frost. No significant correlation between germination percentage and glutamic acid decarboxylase activity was found, but the correlation involving topographical TTC-test was high ($r = +0.996^{***}$).

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SPECIFIC ROTATION OF CEREAL AND LEGUME STARCHES[1]

R. D. PATEL, R. P. PATEL, AND R. S. PATEL

ABSTRACT

Defatting starch with 85% methanol decreased the protein content considerably. The average specific rotation values for sixteen defatted cereal and legume starches were 203° and 202° respectively. The extreme range of specific rotation values, calculated on the basis of true starch content determined by difference and by enzyme-acid hydrolysis, was 201°–204°. Genetic factors and growth conditions have thus little influence on this property of starch.

This investigation was undertaken with the purpose of assessing the improved calcium chloride polarimetric procedure (2,3) as a means of determining starch in cereal and legume starch products. Specific rotation values for sixteen different cereal and legume starch samples of high purity have been determined under the conditions of this method.

Materials and Methods

The grains were soaked in a 0.3% sulfur dioxide solution and crushed to pulps. The starches were isolated from the pulps by the procedure of Clendenning and Wright using 0.25*N* sodium hydroxide solution instead of 0.25*N* ammonium hydroxide solution to render the proteins more soluble. The starches were then extensively extracted by five 2-hour digestions with 85% methanol (5) under reflux. They were filtered and washed with 85% methanol between each extraction and were finally extracted for 8 hours with 85% methanol in a Soxhlet. (This treatment eliminates the fats and reduces the protein.) The starches were dried in the sun, ground, and passed through an 80-mesh sieve.

The moisture contents of the samples were determined by drying to constancy *in vacuo* (10–15 mm. of mercury) at 105°C.; ash by ignition at 575°C.; and protein by Kjeldahl procedure (1). A protein conversion factor of 5.7 was employed in calculating the protein content of wheat starch, 6.25 being used for all others.

The true starch content of all the samples was determined by subtracting the measured content of moisture, ash, and protein from 100 (Table I, column A) and also by the diastase-hydrochloric acid meth-

[1] Manuscript received August 18, 1958. Contribution from the Department of Chemistry, Sardar Vallabhbhai Vidyapeeth, Vallabh-Vidyanagar, Anand, India.

od (1). A dextrose-starch conversion factor of 0.93 was used with all the starches.

The starch solutions were prepared for polarimetry by the method of Clendenning and Wright (3). Ten milliliters of 5% uranyl acetate were included as protein precipitant (2). The filtrate was polarized in a 20-cm. tube, using sodium light. Defatted starches provide clearer solutions and the polarimetric field is more sharply defined than with nondefatted starches.

Results and Discussion

Sixteen specific rotation values for cereal starches range only from 202.2° to 204°, averaging 203° (Table I). Observations on cereal starches in India agree closely with those of Earle and Milner (4) in

TABLE I
SPECIFIC ROTATION OF DEFATTED CEREAL AND LEGUME STARCHES

STARCH SOURCE: GENUS AND SPECIES, AND ENGLISH EQUIVALENT	MOISTURE	ASH	PROTEIN	SPECIFIC ROTATION	
				A	B
	%	%	%	*degrees*	*degrees*
CEREALS					
Eleusine coracana Gaerth. (Raggee millet)	7.80	0.03	0.33	203.0	203.4
Paspalum scrobiculatum L. (India pappusgrass)	7.80	0.03	0.39	202.6	202.8
Triticum sativum Law. (wheat)	7.70	0.07	0.35	202.7	203.3
Oryza sativum L. (rice)	7.50	0.05	0.33	202.2	204.0
Andropogon sorghum Brot. (sorghum vulgare)	7.50	0.08	0.29	203.1	203.5
Pennisetum typhoideum Rich. (pearl millet)	7.65	0.05	0.40	202.5	203.1
Panicum miliare Lamk. (little millet)	7.60	0.07	0.35	202.8	202.2
Panicum miliaceum L. (broomcorn millet)	7.60	0.05	0.39	202.8	203.2
LEGUMES					
Lathyrus sativus L. (grass peavine)	8.48	0.06	0.30	201.9	202.2
Dolichos lablab L. (hyacinth bean)	8.02	0.08	0.25	201.8	203.0
Cajanus indicus Spreng. (pigeon pea)	9.90	0.04	0.21	202.4	203.0
Phaseolus mungo L. (urd bean)	12.00	0.06	0.15	201.4	201.8
Cicer arietinum L. (gram chick pea)	12.30	0.03	0.27	201.0	201.0
Phaseolus aconitifolius Jacq. (moth bean)	10.10	0.08	0.30	201.6	202.8
Vigna catjang Endl. (Catjang cowpea)	10.80	0.06	0.30	202.3	203.0
Pisum sativum L. (garden pea)	11.40	0.02	0.30	202.2	203.0

the United States and with those of Clendenning and Wright (3) in Canada. For this reason it is concluded that the specific rotatory power of cereal starches is not influenced significantly either genetically or by the particular environment under which the starch is synthesized.

Sixteen specific rotation values for legume starches (Table I) have slightly lower average than for cereal starches (202° instead of 203°). Legume starch values are appreciably higher than the values for legume starches reported by Clendenning and Wright (3). These small differences may have resulted from different contents of impurities or inaccuracies in their estimation. Since average specific rotation values for legume and cereal starches agreed within 0.5%, it appears likely that the specific rotatory power of starches from cereals and legumes is actually the same.

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METHODS FOR MEASURING REACTIVITY OF CHEMICAL LEAVENING SYSTEMS
I. Dough Rate of Reaction[1]

JOHN R. PARKS[2], AVROM R. HANDLEMAN[3], J. C. BARNETT[3], AND F. H. WRIGHT[3]

ABSTRACT

The reaction rate of a chemical leavening system in a specified biscuit dough is measured manometrically during mixing by a standardized procedure. Operations of the apparatus are electronically programed. Small pressure increases (resulting from evolution of leavening gas) are plotted as percent of total leavening against time, on a strip chart recorder.

Flour differences, flour age, and the presence or absence of milk and shortening influenced test results. Differences in geometry of the closed system, stirring rate, and test temperature also significantly affected the test values.

In the procedure employed with sodium acid pyrophosphate, the standard error of single determinations was 0.5; with anhydrous monocalcium phosphate the standard errors were 0.7 and 1.2 for 2- and 10-minute measurements respectively.

Although about 100 million pounds of leavening acids are consumed each year in self-rising flours, commercial and household baking powders, prepared mixes, and refrigerated bakery products, very little has been published concerning their mode of action. It is the purpose of this series of papers to describe methods for measuring reactivities of the important leavening chemicals and the effects of reactivity on quality of the leavened product.

Over-all quality of chemically leavened products may be affected by leavening during mixing, holding or handling prior to baking, and baking or frying. Knowledge of the reactivities of leavening systems is used in product development and in specifying performance characteristics of certain leavening acids.

The measurement of reactivity is important for control purposes where the leavening agent can vary in rate characteristics, especially when a particular reactivity range is required. Two such adjustable leavening acids are stabilized anhydrous monocalcium phosphate (AMCP) and sodium acid pyrophosphate (SAPP).

A satisfactory procedure for measuring reactivity must provide

[1] Manuscript received October 5, 1959. Contributed by Research Department, Inorganic Chemicals Division, Monsanto Chemical Co., St. Louis 66, Mo.

[2] Applied Mathematics Section, Research and Engineering Division.

[3] Respectively: Research Department, Technical Service, and Research Department, Inorganic Chemicals Division.

1) a reliable means for quantitatively measuring gas formation, and 2) a standardized method for combining the reactants and specified dough ingredients. Very close temperature control is mandatory for comparable results, and all methods involve maintaining the dough container at constant temperature.

Several different principles have been employed to follow the evolution of gas into and from test doughs or batters. Although these techniques were usually employed with yeast systems, most would be applicable to chemical leavening systems as well.

Pressure measurements employing mercury manometers (7,30) or liquid-filled Bourdon tube gages (24) attached to sealed dough containers have long been used. Solutions of brine and sodium hydroxide have been included, on occasion, to control humidity and to differentiate between retained and expelled gas respectively (16,35). Pressure-recording devices have been developed for manometers (35) and for use with electronic pressure transducers (29).

Constant-pressure gas production has been followed with different types of gas burets (11,31,32). Bailey (2) modified a volumetric device to estimate gas pressure within a fermenting dough. Weight-recording devices, which sense gas evolution by changes in the buoyancy of a system, have been employed (8,12,14). The relative effectiveness of these techniques has been evaluated by several workers (11,15,31).

Amount of liquid expelled from a closed rigid system has been used to indicate gas production (20,27,28). Modifications of this principle to permit measurement at constant pressure have been described (12,17,18,23,25).

Sequential carbon dioxide analyses of chemically leavened doughs[4] (21) have been employed to follow leavening reaction during baking. Simultaneous measurements of dough expansion and carbon dioxide analysis of the atmosphere above the dough were employed to follow retained and evolved gas during fermentation.

Special techniques have been developed to estimate (26) and to measure (19,22) the instantaneous rate of gas evolution. Tracer techniques have been used to study carbon dioxide diffusion rates (9). The possible use of C^{14} sodium bicarbonate as a guide for following gas evolution from chemically leavened systems has been discussed in this laboratory, but technical difficulties in counting beta-radiation limit that approach at this time.

An apparatus for measuring gas production in a chemically leav-

[4] Handleman, A. R., and Joslin, R. P. Determination of baking rate of reaction by means of the Chittick gasometer. Presented at the 41st annual meeting, New York City, May 1956.

ened batter or dough was described by Barackman (6). This device employed a metal bomb fitted with a stirring device, the shaft of which passed through a mercury seal to prevent leaks. Gas production was detected in a buret (10). Comparative data on several leavening acids were reported by Van Wazer and Arvan (34), who used a similar device. In these devices, water is added to dry dough or batter ingredients after the system is closed.

Standardized methods for measuring the reactivities of sodium acid pyrophosphate (SAPP) and anhydrous monocalcium phosphate (AMCP) during dough mixing are described in this paper. These methods were developed to control the uniformity of these products or their various grades. The methods are described in detail, along with sources of error and estimates of precision. Principles of apparatus design are outlined. Design details and working drawings can be obtained by writing to one of the authors[5].

Materials and Methods

The factors to be considered in measuring the dough rate of reaction (DRR) of a chemical leavening system include control of temperature, a uniform method of combining reactants, maintenance of a constant environment, and provision of means for detecting the extent of reaction as a function of time. The apparatus and procedure described below were designed to provide control over these factors and to minimize time required per measurement for the technician.

In essence, the test consists of 1) preparing a complete, dry dough mix; 2) adding a standard amount of water; 3) carrying out a standard mixing and holding cycle in a closed system; and 4) measuring gas production as a function of time. Whether the gas remains in the dough or leaves it is of little consequence in measuring the *reactivity* characteristics of the leavening acid.

Apparatus for Dough Rate of Reaction. The apparatus used senses very slight pressure changes electronically by means of a linear transducer. As an aid to reproducibility and simplicity of operation, the apparatus was programed to carry out all the steps of a determination automatically. Figure 1 is a photograph of the DRR machine, and Fig. 2 is a block diagram showing the essential components, which are 1) a reaction bomb fitted with a stirring mechanism; 2) a thermostatted bath (held at 27±0.01°C.) in which the bomb can be immersed; 3) a mechanism for adding a predetermined amount of water; 4) ballast tanks in the gas-receiving system to make changes in pressure small; 5) a linear pressure transducer connected to an electronic

[5] J. C. Barnett.

recorder capable of being adjusted for zero, millivolt span, and sensitivity; 6) a programing circuit to control the sequence of operations; and 7) a large air reservoir on the low-pressure side of the transducer

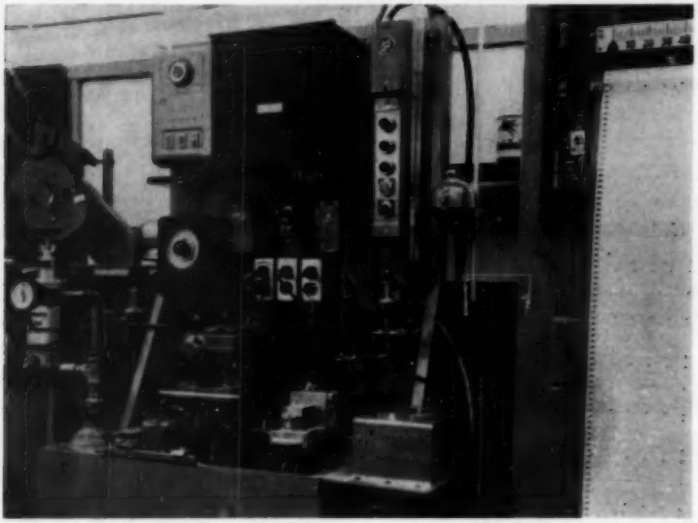

Fig. 1. Programed apparatus for measurement of dough reaction rate.

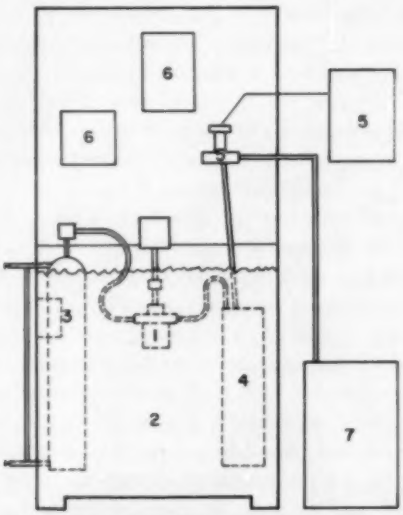

Fig. 2. Block diagram of programed apparatus for measurement of dough reaction rate. Numerals designate components described in test.

for isolating the system from the atmosphere during a test, to eliminate the effects of slight changes in atmospheric pressure. The pressure transducer-recorder system is calibrated to plot percent of total carbon dioxide evolved as a function of time.

The program is arranged 1) to provide a 5-minute delay for bomb and reactants to come to bath temperature; 2) to add the predetermined volume of water; 3) to close the system; 4) to stir for the standard time (3 minutes for SAPP and 10 for AMCP); 5) to keep the system closed until the cycle is complete (8 minutes for SAPP and 10 for AMCP); and then 6) to open the system and return controls to the start position. These operations could be carried out manually and gas evolution detected by other means so long as the equipment is calibrated. The electronic pressure-sensing system permits measurement of gas evolution with only slight changes in pressure on the dough (12 in. of water), but a mechanical pressure-measuring device might be employed, if larger pressure changes are shown not to bias results.

Figure 3 shows the bomb and stirring mechanism. For the work reported below, a stirring rate of 120 r.p.m. was used unless otherwise specified.

Fig. 3. Bomb and stirrer of apparatus for measurement of dough reaction rate.

Dough Ingredients and Laboratory Apparatus. Ingredient specifications and amounts used in doughs as employed for sodium acid pyrophosphate (SAPP) and coated anhydrous monocalcium phosphate (AMCP) are presented in Table I.

Laboratory Apparatus. Equipment required other than normal laboratory apparatus and the DRR machine itself is 1 table fork; 1 mixing bowl, 120–125-mm. diameter at top (an evaporating dish is

TABLE I
COMPOSITION OF TEST MATRICES

Ingredient	Specification	Amount Used	
		Sodium Acid Pyrophosphate Matrix	Anhydrous Monocalcium Phosphate Matrix
		g	g
Flour	Highly chlorine-bleached (pH 4.8–5.0) low-protein angel food cake flour (12.5% moisture basis)	57.0	57.0
Nonfat dry milk	High-heat spray-dried	5.0	5.0
Shortening	Hydrogenated vegetable	6.0	0
Salt	Reagent grade sodium chloride	1.0	0
Soda	Granular sodium bicarbonate (about 5% retained on 100-mesh and about 5% through 270-mesh)	0.755	0.755
Leavening acid	Test	1.057	0.904
Water	Distilled, held at run temperature (27°C.)	40.0	40.0

satisfactory); two 16-oz. wide-mouth, screw-cap glass jars; flour sifter; 1-gal. wide-mouth jar.

Test Procedure. Sift flour for a single day's tests four times and store in a 1-gal. screw-cap jar. Weigh dry ingredients other than shortening and place in 16-oz. bottle. Weigh flour and nonfat dry milk first and agitate sufficiently to partially blend before adding other materials. Blend dry ingredients by tumbling for 3 minutes, tapping the top and bottom of the jar on laboratory bench to free adhering material. In testing SAPP, place the blended dry ingredients in the evaporating dish and cut in the shortening with 50 downward strokes of the fork, using the edge rather than the flat. Clean the fork on the side of the bowl and repeat.

Place the resulting dry mixture in the bomb and attach to the DRR apparatus. Start the program already described. The DRR value of SAPP is defined as the 8-minute reading. For AMCP the 2- and 10-minute readings are taken.

The results with this programed machine were slightly higher than those obtained with a basically similar, manually operated device in which gas production was measured volumetrically with a Chittick gasometer (34). The results by the two machines were highly correlated; the differences in the absolute values are attributed to stirring rate and the dimensions of bomb and stirrer. AMCP results are corrected to correspond to results which would be obtained with the old machine in the data presented below, but SAPP results are presented as observed.

Typical Results

Typical DRR curves obtained with monocalcium phosphate monohydrate ($MCP \cdot H_2O$) and AMCP are shown in Fig. 4. The standard dough formula and procedure developed for AMCP were used in obtaining these curves. Values for 2- and 10-minute DRR, taken from these curves, are presented in the table below. The curve for

Test Results with AMCP

Sample No.	*2-minute DRR*	*10-minute DRR*
1	22.5	72.0
2	26.5	74.5
3	37.0	72.0

sodium bicarbonate alone shows the amount of reaction to be expected from the acidity of the dough constituents. The test procedure provides a means for selecting or specifying materials with desired characteristics such as AMCP's 1 and 2, which are typical of the extremes of good commercial products. Materials with limited delay, such as AMCP 3, can be rejected, thereby reducing product variation due to leavening characteristics. The test is of little value when applied to

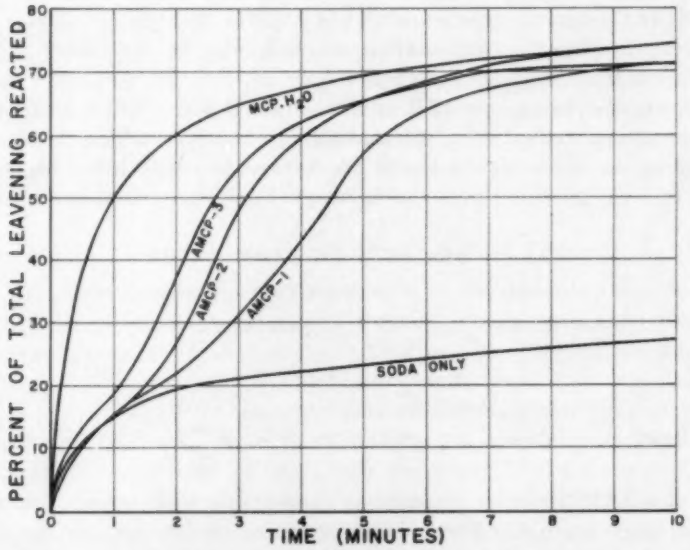

Fig. 4. Dough reaction rate curves obtained with anhydrous monocalcium phosphate (AMCP) and monocalcium phosphate monohydrate ($MCP \cdot H_2O$).

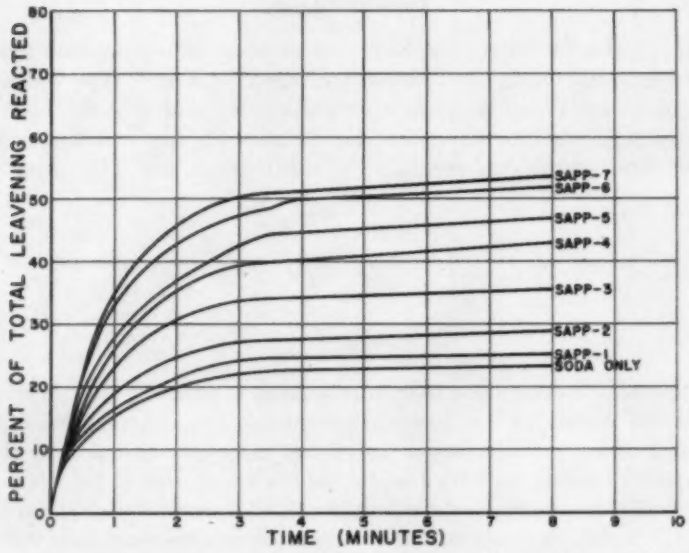

Fig. 5. Dough reaction rate curves obtained with sodium acid pyrophosphates (SAPP's) of varying reactivity.

$MCP \cdot H_2O$, because this material has little or no delay as shown by the curve, which is characteristic for this material and shows little variation from sample to sample.

A number of typical DRR curves obtained with SAPP's of a wide range of reactivities are illustrated in Fig. 5. DRR values (8-minute reading) for these materials and for the test in which leavening acid was omitted are listed in the table below. From these data, it is appar-

Test Results with SAPP's

Sample No.	*DRR Value (8-minute reading)*
1	(Soda only) 25.0
2	28.5
3	35.5
4	42.5
5	46.5
6	52.0
7	54.0

ent that SAPP's can be prepared so as to give a wide range of dough reactivities. Since SAPP's of several different reactivities are commercially available, it is possible to specify a material with reactivity most suitable for a specific application on the basis of its DRR value.

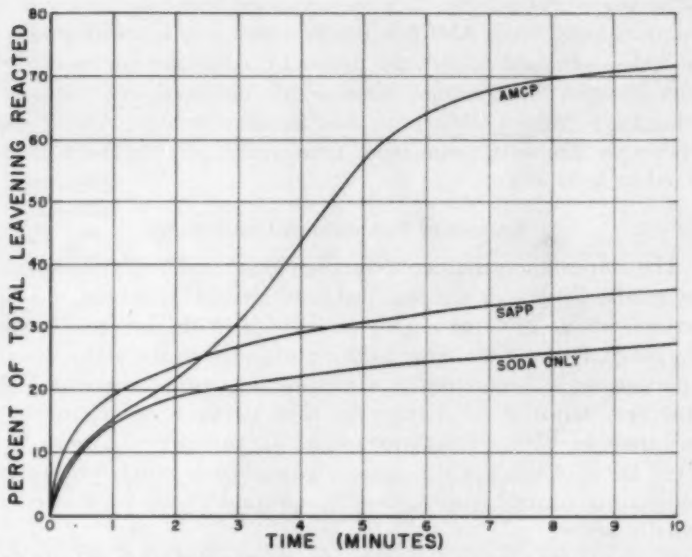

Fig. 6. Comparison of delayed leavening characteristics of sodium acid pyrophosphate (SAPP) and anhydrous monocalcium phosphate (AMCP) determined by the AMCP method.

In Fig. 6, the AMCP standard procedure is used to illustrate the basic difference between the modes of action of AMCP and SAPP. It is apparent that the delay in AMCP is quite temporary, whereas that of SAPP is of considerably longer duration. Both react to near completion in the oven (see footnote 4). Selection again depends on product requirements.

Precision of Dough Reaction Rate Test

The SAPP standard procedure yielded results with a standard error ± 0.5 for single determinations. This value was obtained from 25 determinations with one sample and no change in ingredient supply. The tests were made by five different operators on five days during a 3-week period. Other measurements made before and after this test agree well with this value. Since SAPP's are commercially available in a DRR range from 24 to 43, this test is capable of distinguishing several classes within the range.

Results obtained with the AMCP procedure had standard errors of ± 0.7 for the 2-minute value and ± 1.2 for the 10-minute value. These values were obtained from 39 determinations with no change

in the ingredient supply. The values agree with previous and later determinations. Since AMCP is not available in a series of grades, the test results are used to indicate product quality and uniformity. The lower limit of the 2-minute value is that obtained when sodium bicarbonate is reacted without an acid leavener present. AMCP should not have a 2-minute value more than a few percent above that obtained for soda alone.

Sources of Variation in Test Results

The important variables have been examined for their effects on test results. Although temperature data are not presented, the DRR increases about 10% per degree Centigrade in the practical temperature range. Further, the large ballast tanks used in the above-described apparatus to maintain nearly atmospheric pressure make this apparatus very sensitive to changes in bath temperature during a run. Variations in bath temperature of 0.1°C. produce variations in observed DRR of 0.9. For this reason, a large bath with a very sensitive temperature control is used. Other important effects are treated individually below.

Flour. The flour used in this test is specified as highly chlorine-bleached angel food cake flour, because it is thought that such flour shows a relatively small change in acidity on storage. Nevertheless, differences between flours and changes during storage affect the DRR test. Table II compares the 2-minute and 10-minute DRR values obtained with three different batches of flour. Results obtained

TABLE II
Effect of Flour on Test Results

Flour Sample[a]	DRR Values Obtained with Sodium Bicarbonate But No Acid Leavener by AMCP Procedure	
	2-Minute Value	10-Minute Value
A	27.4	35.5
B	24.5	32.2
C	23.2	31.6

[a] Average of 4, 15, and 28 determinations, respectively.

with flours B and C do not differ significantly, but flour A differs from flour C by about 4 DRR units and should be discarded. When changing flours, it is necessary to select a new supply which gives results agreeing with those from the old; otherwise, reactivities measured with the different flour supplies will not be comparable.

As flour ages, its effect on the observed DRR changes. In Fig. 7, observed DRR values are plotted against flour age for two flours.

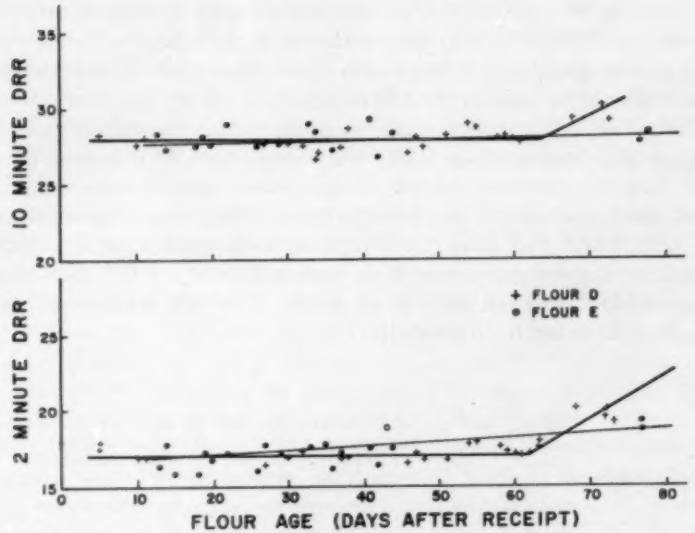

Fig. 7. Effect of flour age on test results with sodium bicarbonate only, for two samples of dough reaction rate (DRR) flour.

While aging caused increased apparent DRR values with both flours, the rate of change for flour D increased abruptly after 60 days, whereas that for flour E continued to increase gradually. The effects of flour variations can be minimized by maintaining a record of DRR obtained with sodium bicarbonate alone and comparing daily results with the average. When the values observed in repeat runs are out of line, it is time to change flour.

Nonfat Dry Milk. Milk exerts a pronounced effect on the behavior of leavening agents. Table III shows that the DRR values for sodium bicarbonate alone are higher in the presence of milk, whereas those for SAPP are much lower in its presence. This difference is explained by the fact that alkaline earth ions supplied by the milk are required for the SAPP delay to be manifested (33). With AMCP, milk is also required for the typical 2-minute delay, as shown in Table IV. In this case, however, milk has an opposite effect on the 10-minute value. The relationship is not yet understood.

Because of their critical effect on observed DRR value, milk solids must be included in test doughs, since the majority of leavened products contain milk.

Variations in the milk effect have been noted with different batches of aged milk solids. For this reason the supply of milk solids should

be stored at 0° to −5°F. New supplies of milk should be checked against the old for test results with the same leavening acid. Only milk giving results which agree with those obtained with the standard supply should be used in the test dough.

Salt. Table IV presents data obtained, with and without salt, for sodium bicarbonate alone and for a complete AMCP leavening system. Salt did not have any significant effect on the results. Earlier DRR work was carried out with the same dough ingredients for testing both SAPP and AMCP. Salt has been dropped from the AMCP procedure for simplification. It is included in the SAPP procedure, because SAPP data comparable to that in Table IV are not yet available, but its value is questionable.

TABLE III

EFFECT OF NONFAT DRY MILK ON TEST RESULTS

SPRAY-DRIED NONFAT DRY MILK	DRR VALUES OBTAINED WITH SODIUM BICARBONATE BY THE AMCP PROCEDURE	
	2-Minute Value	10-Minute Value
Present [a]	24.5	32.2
Absent [b]	18.5	23.1
	With AMCP present	
Present	27.5	75.5
Absent	47.5	70.5
	DRR Values Obtained with Sodium Bicarbonate and SAPP by the SAPP Procedure (8-Minute Values)	
	SAPP-x	SAPP-y
Present	29.2	28.4
Absent	47.1	43.6

[a] Average of 15 determinations.
[b] Average of 4 determinations.

TABLE IV

EFFECT OF SALT ON RESULTS WITH ANHYDROUS MONOCALCIUM PHOSPHATE

REAGENT GRADE SALT	DRR VALUES OBTAINED WITH SODIUM BICARBONATE	
	2-Minute Value	10-Minute Value
	No acid leavener	
Present [a]	23.9	31.5
Absent [b]	24.5	32.2
	AMCP as acid leavener	
Present [c]	33.9	83.5
Absent [d]	32.2	81.4

[a] Average of 7 determinations.
[b] Average of 15 determinations.
[c] Average of 4 determinations.
[d] Average of 2 determinations.

Shortening. The data in Table V suggest a slight decrease in reactivity when shortening is present in the test dough. This effect is attributed to an interference with wetting rather than an influence on the characteristics of the leavening acid being tested. It is thought that omitting shortening has no effect on the relative reactivities observed, and it has been dropped from the AMCP procedure to avoid a possible source of human error and the work of incorporating it. It is still included in the SAPP procedure, however, because data comparable to that obtained with AMCP are not available.

Sodium Bicarbonate. Sodium bicarbonate is available in relatively pure form, and differences between grades are usually related to particle size. By specifying a particle size range, variations due to soda will be avoided.

Machine Differences. Two bombs and two stirrers, originally identical, were used in obtaining the data in Table VI. Stirrer A had become slightly bent and could not be perfectly restored to its former configuration. The data show no difference between the bombs, but there was a distinct difference between results obtained with the two stirrers. The difference points up the importance of geometry, if comparable results between laboratories are desired. A correlation between results obtained with the different stirrers can be established, but

TABLE V

EFFECT OF SHORTENING ON TEST RESULTS

Hydrogenated Vegetable Oil (Shortening)	DRR Values Obtained with Sodium Bicarbonate	
	2-Minute Value	10-Minute Value
	No acid leavener	
Present [a]	23.5	30.5
Absent [b]	23.9	31.5
	AMCP as acid leavener	
Present [c]	32.1	80.7
Absent [c]	33.9	83.5

[a] Average of 7 determinations.
[b] Average of 6 determinations.
[c] Average of 4 determinations.

TABLE VI

EFFECT OF DIFFERENT BOMBS AND STIRRERS ON TEST RESULTS

Stirrer	DRR Values Obtained with Sodium Bicarbonate but no Acid Leavener or Milk by AMCP Procedure			
	Bomb A		Bomb B	
	2-Minute Value	10-Minute Value	2-Minute Value	10-Minute Value
A	20.7	24.5	20.0	24.2
B	18.4	23.4	18.5	22.6

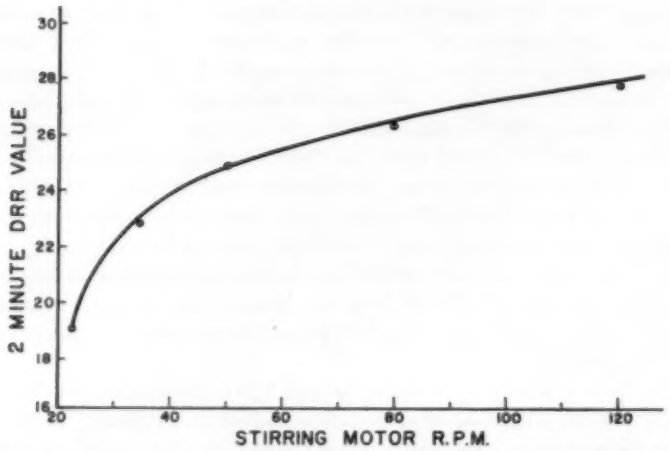

Fig. 8. Effect of stirring rate on 2-minute dough reaction rate observed with anhydrous monocalcium phosphate.

to obtain values which agree, they, and presumably the bombs, must be identical. The gas production sensor can employ nearly any of the principles described in the literature review so long as it is properly calibrated.

Stirring Rate. Figure 8 shows the effect of increased stirring rate on observed DRR. As rate increases, the effect of variations diminishes. The arbitrarily chosen rate of 120 r.p.m. was selected for this equipment, because lower rates gave larger experimental errors and higher rates offered no advantages.

Conclusions

DRR procedures have been used as leavening performance yardsticks for at least 25 years, but their usefulness has been curtailed by lack of standardization of methods among laboratories. It was possible, in a given laboratory at a given time, to compare the reactivities of leavening acids, but results obtained at a later date or in another laboratory were not comparable.

By standardizing test ingredients and test procedure and by correlating DRR equipment with a given reference apparatus, it is possible to obtain comparable results in different laboratories. To establish the relationship between two DRR machines, measurements should be made on several leavening acids of different reactivities with the two machines.

The importance of more exact definition of leavening reactivity

characteristics is being heightened by the development of new products with more stringent leavening requirements and less tolerance for variation. The DRR test described in this paper has proved satisfactory for this purpose in routine commercial practice.

Acknowledgments

The authors wish to acknowledge the contributions of a large number of other workers in this field, the results of whose efforts and ideas are embodied in this paper. Included among these are Robert P. Langguth, Robert P. Joslin, Thomas P. Kichline, J. Walter Carlson, Henry V. Moss, Robert E. Scherer, Clayton F. Callis, Robert A. Miller, Elizabeth McKim, and Miriam Lenz. The authors also wish to express their appreciation for the helpful guidance rendered by John R. Van Wazer.

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DETERMINATION OF THE EXTENT OF REACTION BETWEEN EPICHLOROHYDRIN AND STARCH[1]

G. E. HAMERSTRAND, B. T. HOFREITER, AND C. L. MEHLTRETTER

ABSTRACT

The rate and extent of cross-linking of starch with epichlorohydrin was investigated by determining the quantity of unreacted epichlorohydrin in reaction filtrates. The unreacted epichlorohydrin was converted to glycerol by hot alkali. Periodate oxidation of the glycerol solutions yielded formaldehyde, which was estimated colorimetrically using the chromotropic acid color reaction.

Under the reaction conditions described a maximum extent of reaction of 90% was obtained. A blank correction was required to account for alkali-solubilized material from starch, which under the conditions of analysis yielded an apparent value for epichlorohydrin. It was required, for valid application of these analyses, that the epichlorohydrin-starch reaction be conducted in a gas-tight system. The quantities of epichlorohydrin reacted, as indicated by the analyses, are not necessarily exclusively involved in cross-linking. It was found that with molar ratios of anhydroglucose units to epichlorohydrin as low as 1,200 to 1, marked inhibition of the granule to swelling in hot water resulted.

Epichlorohydrin has been extensively used to produce inhibited or cross-linked starches (1,6). In its reaction with hydroxyl groups of starch, mono- and diethers are formed, the diethers being either inter- or intramolecular. From this complex mixture the exact amount of cross-linking achieved by intermolecular diether formation would be difficult to ascertain. Generally, the extent of cross-linking is calculated on the basis of total epichlorohydrin introduced into an alkaline aqueous starch slurry, or it is qualitatively determined from the physical behavior of the pasted products (5). A more reliable estimate of the degree of cross-linking, based on the epichlorohydrin that has reacted with the starch, would be of practical interest to the starch industry.

The reaction of epichlorohydrin with starch was investigated in order to define more reliably the extent of cross-linking. This study entailed developing a procedure for accurately determining unreacted epichlorohydrin in the reaction mixtures.

Under the alkaline conditions required for the cross-linking of starch, partial hydrolysis of epichlorohydrin to glycerol occurred. The result was to preclude applying the method of Daniel and Gage (3) which is specific for epichlorohydrin. The rate and extent of reaction

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of epichlorohydrin with starch were determined by converting excess epichlorohydrin to glycerol, which was estimated spectrophotometrically by the chromotropic acid method (7) after periodate oxidation to formaldehyde (8).

Materials and Methods

Reagents. The commercial pearl corn starch that was used contained by analysis: methanol extractables, 0.63%; ash, 0.08%; nitrogen, 0.05%; and moisture, 9 to 12%.

Stock alkali solution was prepared by dissolving 0.66 g. of reagent grade sodium hydroxide and 16.66 g. of reagent grade anhydrous sodium sulfate in 100 ml. of distilled water.

Epichlorohydrin obtained from the Shell Chemical Corporation[2] was redistilled before use (b.p. 114.5°–116.5°C.). A solution was made by dissolving 0.196 ml., measured from a calibrated microburet, in 50 ml. of the stock alkali solution. A 5-ml. aliquot of this solution was diluted to 200 ml. with distilled water and contained 116 μg. of epichlorohydrin per ml.

Glycerol (Eastman White Label) had a purity of 95.0% by specific gravity. A solution was prepared by dissolving approximately 250 mg. in 50 ml. of the stock alkali solution. A 5-ml. aliquot of this solution was diluted to 200 ml. with distilled water and contained approximately 125 μg. of glycerol per ml.

The following standard solutions were prepared with analytical or C.P.-grade reagents: 0.1*M* sodium metaperiodate, 1.0*M* sodium arsenite, and 10*N* sulfuric acid.

Chromotropic acid (disodium 4,5-dihydroxy-2,7-naphthalenedisulfonate) in the amount of 1.13 g. was dissolved in 100 ml. distilled water and then filtered. To this were added 450 ml. of a sulfuric acid solution prepared by adding 300 ml. of C.P. sulfuric acid to 150 ml. distilled water. The chromotropic acid should be prepared fresh every 2 or 3 weeks. Exposure of the solution to direct sunlight should be avoided.

Anthrone reagents were prepared by dissolving 0.1 g. (Eastman White Label) in 50 ml. of concentrated C.P. sulfuric acid.

Reaction of Epichlorohydrin with Starch. Reaction conditions were essentially those described by Caldwell (2), with the exception that a gas-tight system was employed to prevent escape of volatile epichlorohydrin. One hundred grams (dry basis) of starch were suspended in 150 ml. of stock alkali solution by mechanical stirring. The

[2] The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

desired quantity of epichlorohydrin (20–900 mg.) was then freshly dissolved in 50 ml. of stock alkali solution and added dropwise to the starch mixture in 3-5 minutes. After 18 hours at 25°C. the slurry was neutralized to pH 6.0 with 6*N* sulfuric acid and filtered. The starch was resuspended in distilled water (20% starch by weight), then filtered, and the two filtrates were combined and analyzed for unreacted epichlorohydrin. Two more washes, used in the isolation of cross-linked starches, had been shown to contain only 1.8% of the total recoverable epichlorohydrin and were therefore routinely omitted from the analysis.

Determination of Unreacted Epichlorohydrin. An aliquot (5–25 ml.) of the combined filtrates containing 500–750 μg. epichlorohydrin was pipetted into a 50-ml. volumetric flask and 1 ml. of 2*N* sodium hydroxide was added. An epichlorohydrin standard and a reagent blank were prepared in a similar manner. The stoppered flasks were heated on a steam bath for 1 hour, then cooled, and 1 ml. of 10*N* sulfuric acid was added, followed by 5 ml. of 0.1*M* sodium periodate. The flasks were placed in the dark for 10 minutes, after which 5 ml. of 1.0*M* sodium arsenite were added and the solutions diluted to 50 ml. with distilled water. One-milliliter aliquots were then pipetted into matched spectrophotometer tubes. Ten milliliters of chromotropic acid reagent were added rapidly with mixing to each of the tubes, which were then heated in a boiling-water bath for 30 minutes. The tubes were removed and cooled to room temperature, and the absorbance was read at wave length of 570 mμ in a Coleman Jr. Spectrophotometer using a water blank set at zero absorbance. Concentrations of the samples were calculated using absorbance and the instrument constant. Absorbance of the solutions obeyed the Beer-Lambert Law.

Solubility of Cross-Linked Starches. Solubility of the cross-linked starches in hot water was determined in the following manner: 0.800 g. (dry basis) of the reacted starch was weighed into a graduated 40-ml. centrifuge tube, and distilled water was added to the 40-ml. mark. The mixture was heated in a boiling-water bath for 30 minutes with occasional stirring as required to prevent settling. After cooling to room temperature the stirring rod was removed, distilled water was added to bring the volume to 40 ml., and the mixture was centrifuged at 3,000 r.p.m. for 10 minutes. Total solids content of the supernatant was determined by the anthrone method for carbohydrate material (4).

Results and Discussion

Blank Correction. The small quantity of soluble carbohydrate

material (approximately 0.04% by weight) in the filtrates from the reaction mixtures interfered with the epichlorohydrin analysis by producing formaldehyde on treatment with periodate. The correction for soluble carbohydrate was determined by treating corn starch in the same manner as the reacted starch but in the absence of epichlorohydrin. For one lot of corn starch a correction equivalent to 16.3 mg. apparent epichlorohydrin for each 100 g. of starch was obtained with a coefficient of variation of 2.65% in ten separate determinations. A different lot of starch, however, was found to have an average value of 21.5 mg. of apparent epichlorohydrin per 100 g. of starch. Appropriate correction was made for each lot of starch reacted.

Experimental evidence for the validity of the blank correction was obtained in the following manner: 200 g. of corn starch were dispersed in 300 ml. of the stock alkali solution by mechanical stirring for 48 hours at 25°C. The starch was filtered, washed with distilled water, and divided into two equal portions. One portion, approximately equivalent to 100 g. of starch, was reacted with epichlorohydrin in the normal procedure for 18 hours. A parallel trial without epichlorohydrin was made with the other portion. The blank correction was 2.5 mg. of apparent epichlorohydrin per 100 g. of starch. The percentage epichlorohydrin which had reacted with the starch, however, was identical with that found in a comparable experiment with the same starch not pretreated with alkali. The correction for the untreated starch was 16.3 mg. of apparent epichlorohydrin per 100 g. of starch. Similar results were obtained with various mole ratios of starch and epichlorohydrin.

Rate of Reaction. The rate and extent of reaction of epichlorohydrin and starch were determined by removing aliquots from the reaction mixture at various intervals of time. The supernatants were

TABLE 1

RATE OF REACTION OF STARCH WITH EPICHLOROHYDRIN AT 25°C.

Reaction Time	Unreacted Epichlorohydrin			Epichlorohydrin Reacted[a]	Reacted	Calculated Cross-Linking
	Total	Blank	Corrected			
hours	*g*	*g*	*g*	*g*	%	*AGU/C.L.*
0.5	0.377	0.050	0.327	0.135	29.2	2,050
1.0	.375	.053	.322	.140	30.3	1,980
2.0	.338	.063	.275	.187	40.5	1,480
4.0	.305	.070	.235	.227	49.1	1,220
8.0	.249	.085	.164	.298	64.5	930
18.0	.186	.106	.080	.382	82.7	725
32.0	.174	.119	.055	.407	88.1	680
45.0	0.166	0.120	0.046	0.416	90.0	665

[a] Initially 0.462 g. of epichlorohydrin was added to 485 g. of starch under reaction conditions described earlier.

analyzed for unreacted epichlorohydrin and the isolated samples were pasted, as described previously, to determine changes in solubility. Experimental results from a reaction in which sufficient epichlorohydrin had been added to produce a calculated value of 600 anhydroglucose units per cross-link are shown in Table I. To establish the required blank correction, a similar preparation with no epichlorohydrin added was sampled at the same time intervals. The rate of reaction is shown by the plot of percentage epichlorohydrin reacted at various intervals of time (Fig. 1). The heterogeneous nature of the reaction system and surface adsorption effects would complicate a quantitative treatment of the kinetics of this reaction.

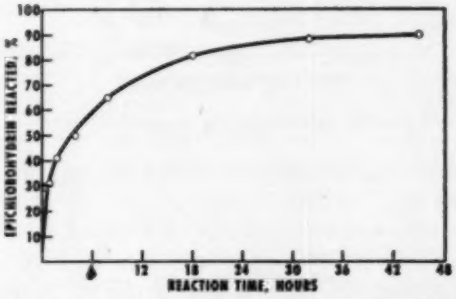

Fig. 1. Rate of reaction of epichlorohydrin with starch at 25°C.

At levels of reaction ranging from 2,500 to 2,550 anhydroglucose units per cross-link it was found that an average of 78.2% (coefficient of variation of 2.2%) of the quantity of epichlorohydrin initially added reacted with the starch in 18 hours. Representative data are given in Table II.

TABLE II

REACTION OF EPICHLOROHYDRIN WITH 100 GRAMS OF STARCH FOR 18 HOURS AT 25°C.

Theoretical Cross-Linking	Epichlorohydrin Added	Unreacted Epichlorohydrin			Epichlorohydrin Reacted	Reacted
		Total	Blank	Corrected		
AGU/C.L.	*g*	*g*	*g*	*g*	*g*	%
50	1.140	0.288	0.0214	0.267	0.873	76.6
100	0.588	.143	.0215	.122	.466	79.3
247	.231	.0717	.0165	.0552	.1758	76.1
482	.1180	.0397	.0168	.0229	.0951	80.6
800	.0714	.0382	.0220	.0162	.0552	77.3
960	.0595	.0273	.0163	.0110	.0485	81.5
1,600	.0357	.0237	.0160	.0077	.0280	78.4
2,360	0.0241	0.0212	0.0163	0.0049	0.0192	79.7

Hot Water-Solubles. A near linear relationship exists between the quantity of hot water-solubles and the amount of epichlorohydrin that had reacted with the starch expressed in terms of anhydroglucose units per cross-link. Figure 2 shows that hot water-solubles can be a

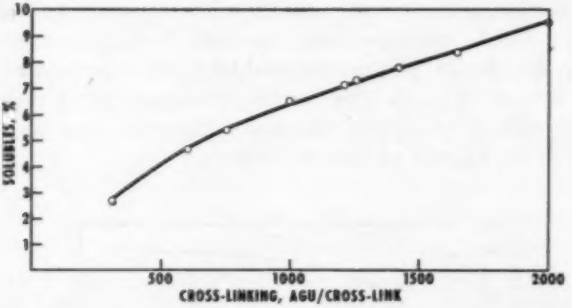

Fig. 2. Relation of solubles to cross-linking in epichlorohydrin-reacted starch.

dependable measure of the relative extent of cross-linking achieved with epichlorohydrin.

The results presented indicate that in a closed system the extent of reaction of epichlorohydrin with starch can be more accurately determined than in the past. Assuming that the percentage of reacted epichlorohydrin that produces cross-linking in starch is essentially constant throughout the reaction, the method as developed measures the relative extent of cross-linking achieved. Reacted epichlorohydrin and the amount of hot water-solubles in the products are directly related so that the solubles may be used to determine the relative cross-linking effected. A procedure for the determination of epichlorohydrin through conversion to glycerol is described.

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STUDIES ON THE ENDO-BETA-GLUCANASE SYSTEM OF BARLEY MALT[1]

WAYNE W. LUCHSINGER[2], DAVID G. COCHRANE[2], AND ERIC KNEEN[2]

ABSTRACT

Two endo-beta-glucanases were separated from barley green malt. The two enzymes were distinguished by their heat sensitivities, solubilities in ammonium sulfate, and pH optima.

Both enzymes rapidly decreased the viscosity of barley glucan solutions. Neither enzyme was capable of decreasing the viscosity of carboxymethyl cellulose solutions; thus they were distinguished from malt cellulase.

The primary factor in the general softening or physical modification of barley during germination is the breakdown of cell-wall materials. The principal carbohydrate constituents of barley cell walls are cellulose and hemicellulose.

Typically, barley hemicelluloses are mixed hexose-pentose polymers containing glucose, arabinose, and xylose, with perhaps traces of other sugars and sugar derivatives. Glucose polymers isolated from barley hemicelluloses were reported by Aspinall and Telfer (1) to consist of unbranched chains containing approximately equal numbers of beta-1,3- and beta-1,4-linkages. More recent work by Smith's group on glucose polymers isolated from oat and barley hemicelluloses indicates that the -1,3- and -1,4-linkages are not evenly distributed. There are present sequences of -1,3-, and sequences of -1,4-linkages which are separated from one another by much larger molecular segments containing alternating -1,3- and -1,4-linkages (4).

The investigations described in this paper are concerned with the barley malt enzymes which attack the inner linkages of the glucose polymers present in barley hemicellulose. Study of the heat stability of the barley malt endo-beta-glucanase[3] system supported earlier suggestions that there was more than one enzyme responsible for its viscosity-reducing activity (2,5). Lack of a hemicellulose substrate containing only beta-1,3- or beta-1,4-polyglucosidic linkages has been a serious handicap in attempts to determine whether more than one specific polyglucosidase in fact is involved. This difficulty is readily apparent when it is considered that substrates available from barley gums contain both 1,3- and 1,4-beta-polyglucosidic linkages and prob-

[1] Manuscript received November 27, 1959.

[2] Kurth Malting Co., Milwaukee, Wisconsin.

[3] Previously this system was designated endo-beta-polyglucosidase (5). The nomenclature has been changed to conform to that of the substrate, glucan.

ably 1,6 linkages. Hence, viscosity measurements do not distinguish whether the enzyme acting is specific for beta-1,3-linkages, beta-1,4-linkages, for both, or for some other linkage.

The work presented here describes experiments in which heat-stability of the enzymes which hydrolyze barley gums is used to distinguish the types of activity. The heat-stability criterion was used by applying the principle that the heat inactivation of an individual enzyme usually follows first-order kinetics.

Materials and Methods

Enzymes. Crude malt extracts were prepared by mashing finely ground malt for 2 hours in a 0.5% solution of sodium chloride at room temperature. The insoluble material was eliminated by filtration or centrifugation (5). Separation of the activities is described in the text.

Substrate. A 1.72% solution of Kindred barley gum prepared as described previously was used as the general substrate (5). Other substrates used in the investigations are described in the text.

Activity Determination. Enzyme activity was determined by measuring the change in viscosity of the substrate at pH 4.7 and 30°C. in an Ostwald No. 200 viscometer. Endo-beta-glucanase activities were expressed as the change in the reciprocal specific viscosity per 30 minutes per g. of dry malt (5).

Results

Differential Heat-Inactivation of Malt Endo-Beta-Glucanases. A green malt extract was heated at 50°C. for 10, 20, 30, and 60 minutes. The log of the activity remaining after each time interval is plotted in Fig. 1. There was a rapid decrease in activity for the first 20 minutes, after which the rate of inactivation decreased and, as evidenced by the straight-line function, appeared to follow first-order kinetics during the latter portion of the heating. Kinetics of this sort suggest that the green malt extract contained two or more endo-beta-glucanases, one of which was more heat-stable than the others. If this is true, then the heat applied during malt kilning should destroy relatively more of the less stable enzyme.

Figure 2 shows the effects of heating at 40°C. on the endo-beta-glucanase activity of extracts prepared from green malt and from the corresponding kilned malt. The amounts of the more heat-stable activity (A_s) present in the malt extracts can be estimated by extrapolating the straight-line portion of the log curves to zero heating time. The value obtained represents the amount of A_s which was pres-

ent before the heat inactivation was begun. Subtraction of this figure from the initial activity of the extract provides an estimate of the amount of less heat-stable activity (A_L) which was present in the original extract. These values were calculated from the curves in Fig. 2 and are presented in the table below. The results show that

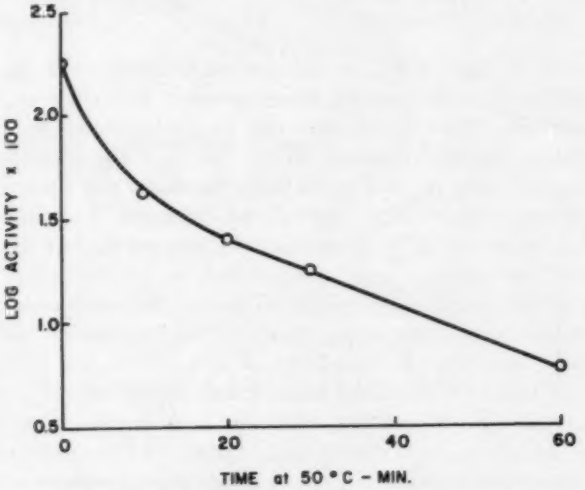

Fig. 1. Heat-inactivation of green malt extract at 50°C.

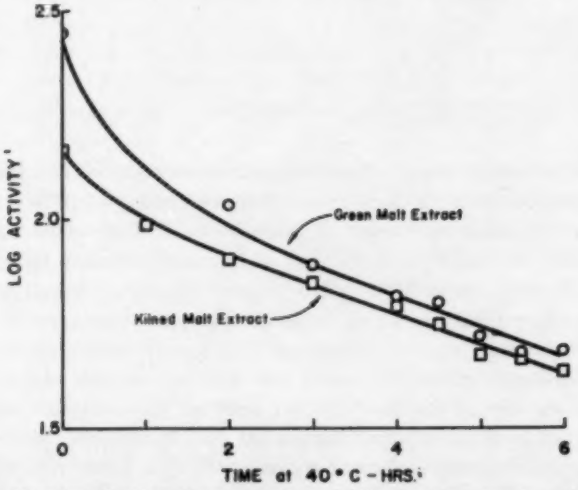

Fig. 2. Heat inactivation of green malt and kilned malt extracts at 40°C.

kilning resulted in a much greater loss of A_L than of A_g. About 90% of A_L was lost during kilning, as compared to about half of A_g.

	Activity	
	Green malt	*Kilned malt*
A_L	11.0	1.3
A_g	9.2	4.8

The data in Figs. 1 and 2 and the table above show that there are two or more endo-beta-glucanases present in barley green malt and in addition, that the activities can be distinguished by their behavior during heat-inactivation. With this tool for identifying the activities, separation of malt endo-beta-glucanases was undertaken.

Separation of Barley Malt Endo-Beta-Glucanases. The classic method of ammonium sulfate fractionation was selected for the separation. In general, the enzymes were precipitated at various levels of ammonium sulfate, followed by dialysis to remove the ammonium sulfate.

The results of a preliminary fractionation experiment are shown in the table below. Nearly one-third of the activity was precipitated between 20 and 40% saturated ammonium sulfate, and two-thirds of the activity between 40 and 60% saturated ammonium sulfate.

Ammonium Sulfate	*Activity of Precipitate*
% saturated	
20	0.1
40	4.9
60	10.4
80	1.2
95	0.0
Original extract	21.1
Original extract dialyzed	17.8

In order to facilitate fractionation and location of the activities it was desirable to omit the rather time-consuming dialysis step. The maximum possible carryover of ammonium sulfate with the enzyme precipitates was 0.27 g. per 100 ml. of reaction solution. Quantities of ammonium sulfate as high as 0.5 g. per 100 ml. of reaction solution were tested and did not affect the endo-beta-glucanase activity.

On the other hand, the results in Fig. 3 show that a small amount of ammonium sulfate did affect the kinetics of the enzyme inactivation. Dilution of the malt extract with an ammonium sulfate solution before heat-inactivation appeared to afford some protection to A_g. The smaller slope of the log curve reflects a lower rate of enzyme inactivation. Dilution of the malt extract with water before heat-in-

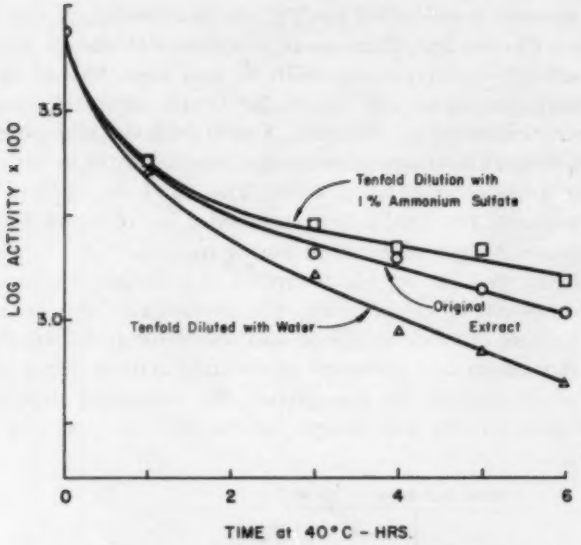

Fig. 3. Effect of ammonium sulfate on the heat sensitivity of malt endo-beta-glucanases.

activation resulted in a more rapid inactivation of A_S, as evidenced by the increased negative slope of the log curve. Even so, all samples provided the same estimation of the amounts of A_L and A_S present, showing the feasibility of directly assaying the ammonium sulfate precipitates.

Enzyme Preparation. The fractionation of a 0.5% sodium chloride extract of barley green malt is presented as a flow diagram in Fig. 4. The insoluble and precipitated materials were removed from the solubles by centrifugation.

Distribution of the endo-beta-glucanase activities in the first five fractions is shown in the table below. Heat-inactivation curves showed that fraction II was 85% A_L and 15% A_S, fraction III was 60% A_L and 40% A_S, and fraction IV was 30% A_L and 70% A_S. Refractionation improved the separation of the activities but resulted in marked

Fraction	*Activity*
	% of total recovered
I	4
II	27
III	36
IV	29
V	4

losses of enzyme. Fraction VII was 95% A_L and fraction X was 85% A_S.

Enzyme Purification. Portions of fractions VII and X were again fractionated. The precipitates which formed from VII at 40% saturated ammonium sulfate, and from X at 60% saturated ammonium sulfate, were collected and dialyzed. For dialysis the precipitates were dissolved in small volumes of water and dialyzed at 0 to 4°C. against 30-volume aliquots of distilled water. The water was replaced at 2.5, 5, and 7.5 hours. The final dialysis proceeded for 16 hours. Containers were subjected to gentle agitation during dialysis.

Very heavy precipitates which formed in both samples during dialysis were removed by centrifuging. The precipitates were resuspended in 0.5% sodium chloride solutions and tested for endo-beta-glucanase activity. Approximately one-tenth of the total activity remaining after dialysis was present in the precipitates; the remaining nine-tenths of the recovered activity was present in the supernatants. The dialysis

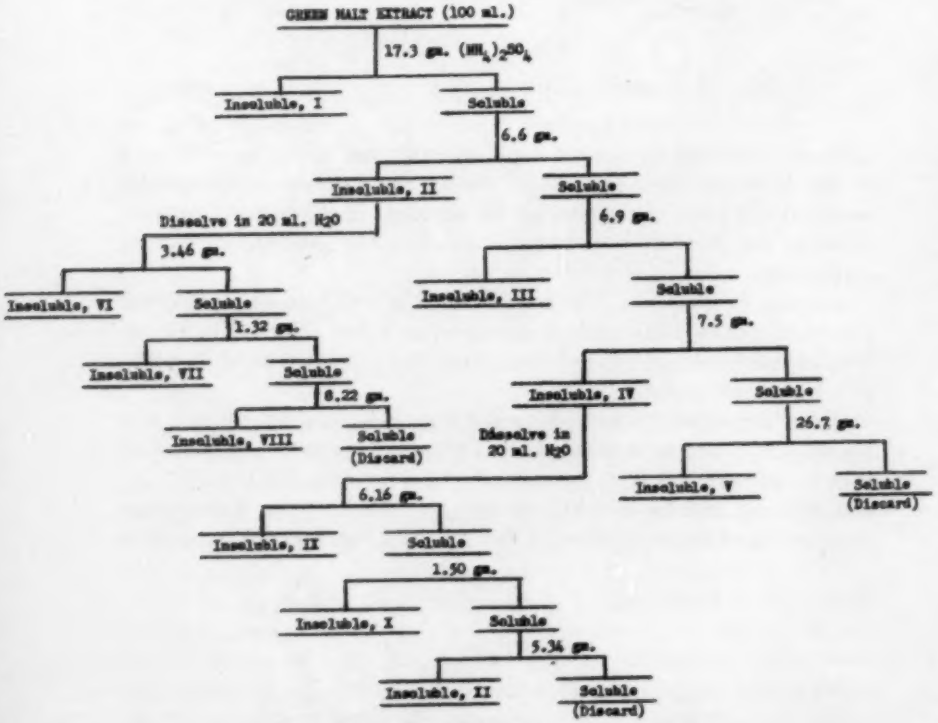

Fig. 4. Flow diagram of the enzyme fractionation.

step resulted in losses of nearly 30% of the initial activities.

The purity of the endo-beta-glucanase present in each supernatant was tested by heat-inactivation. The results are presented in Fig. 5. A_L was 95% free of A_g, and A_g was 90% free of A_L. In addition, the results in Fig. 5 show that A_L was half inactivated by heating for 40 minutes at 40°C. The more heat-stable A_g loses half of its activity

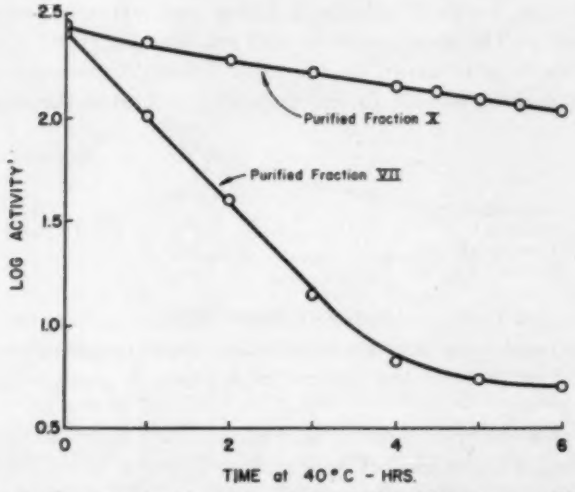

Fig. 5. Heat inactivation of purified endo-beta-glucanases at 40°C.

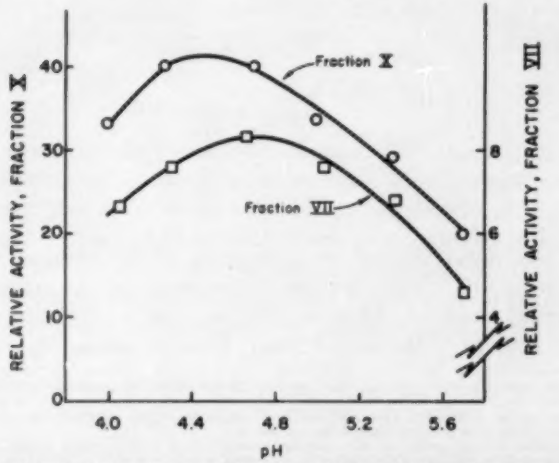

Fig. 6. Effect of pH on activity of purified endo-beta-glucanases.

after being heated for 5 hours and 15 minutes at 40°C.

Effect of pH on Activity. The endo-beta-glucanase activities[4] of fractions VII and X were measured over the pH range of 4.0 to 5.7. pH values reported are those found immediately following the reaction. Replicate experiments, illustrated by the data in Fig. 6, showed that fraction VII exhibited the greatest activity between pH 4.6 and 4.8, and fraction X between pH 4.3 and 4.7.

Studies on Substrate Specificity. Green malt extracts contain cellulase activity[5]. The distribution of malt cellulase in certain ammonium sulfate fractions is shown in the table below. Apparently the malt cellulase is quite soluble in less than 40% saturated ammonium sulfate. Fraction I contained no detectable cellulase activity and fraction VII contained only very little cellulase. Other experiments showed that most of the cellulase present in fraction X precipitates during dialysis.

	Relative Cellulase Activity
Original extract	19.2
Fraction I	0.0
Fraction VII	1.4
Fraction X	5.7

The effect of heating green malt extract for 5 hours at 40°C. on its endo-beta-glucanase and cellulase activity is shown in the table below. During the 5-hour period nearly all the A_L activity and half of the A_S activity was lost (reflected by a decrease of the endo-beta-glucanase activity from 915 to 178). During the same period the cellulase activity remained constant. Thus neither A_L nor A_S contributed to the cellulase activity of the malt extract. That is, neither A_L nor A_S was

Extract Held at 40°C.	*Relative Activity*	
	Endo-beta-glucanase	*Cellulase*
hours		
0	915	18.6
1	318	19.3
2	267	19.4
3	213	19.2
4	191	20.4
5	178	18.4

[4] The results in Fig. 6 and the next two tables are expressed as relative activities. Different amounts of the two enzymes were lost during fractionation and purification; hence, expression of activity on a dry malt basis would be misleading. The activities were compared by adjusting the enzyme solutions to a standard volume and using standard aliquots for the assays.

[5] As reflected by the ability to decrease the viscosity of a carboxymethyl cellulose solution. The activity is expressed as 100 times the change in reciprocal specific viscosity of a carboxymethyl cellulose solution per 30 minutes.

able to attack beta-1,4-linkages distant from the ends of carboxymethyl cellulose molecules.

The action of barley malt extract on a "rye pentose gum" substrate[6] is shown in Fig. 7. The viscosity dropped rapidly from 30 c.p.

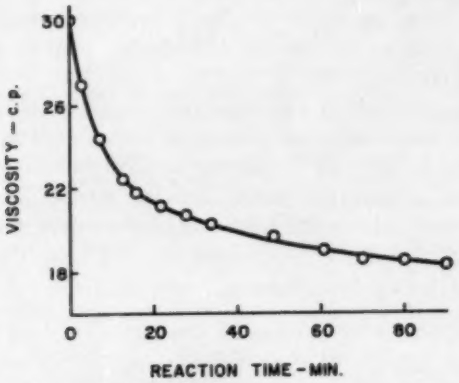

Fig. 7. Effect of barley malt extract on the viscosity of rye pentose gum.

to about 20 c.p., followed by a very slow decrease in viscosity as the reaction continued. The high-molecular-weight carbohydrate[7] which remained after 2 hours' hydrolysis contained 39% arabinose, 54% xylose, 3% glucose, and 4% fructose and/or mannose. It appears that the barley malt extract lacked certain endo-enzymes required to extensively degrade the "rye pentose gum." The same extract rapidly degrades barley glucan to small fragments (as reflected by solution viscosities approaching one).

Discussion

The experiments described herein demonstrated the presence of at least two endo-beta-glucanases in barley malt. One enzyme was considerably more heat-sensitive than the other. Nearly 90% of the more heat-sensitive enzyme was lost during kilning, as opposed to approximately half of the less heat-sensitive enzyme. Thus both enzymes likely are active during germination, while the less heat-sensitive enzyme may play the dominant role during the mashing step of the brewing process.

Preliminary fractionation of the barley malt extracts with more refined procedures has suggested the possible presence of one or more

[6] The "rye pentose gum" contained 18% glucose, 6% fructose and/or mannose, 30% arabinose, and 46% xylose on a weight basis; analyzed per Luchsinger, English, and Kneen (6).

[7] Molecular weights greater than 5,000 to 10,000 on the basis of dialysis experiments (3).

additional endo-beta-glucanases. The recent discovery that barley glucan contains -1,6- in addition to -1,3- and 1,4-linkages (4) likewise may be evidence that the two enzymes described herein do not constitute the entire beta-glucanase system.

The barley malt extracts were unable to extensively hydrolyze rye pentose gums showing a lack of certain endo-pentosanases. Whether a similar behavior would prevail with barley pentose gums has not been determined.

Additional experiments with substrate showed that neither A_L nor A_S contributes to the cellulase activity of barley malt as it is reflected by the decrease in viscosity of carboxymethyl cellulose (CMC). Thus, neither system showed significant activity toward beta-1,4-linkages distant from the ends of the CMC molecules. One might speculate from these results that the two systems also are unable to attack beta-1,4-linkages in barley beta-glucan.

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QUALITY OF THE PROTEIN IN SELECTED BAKED WHEAT PRODUCTS[1]

BARBARA M. KENNEDY AND ADELIA R. SABISTON

ABSTRACT

The quality of protein was determined in baked wheat products containing varying amounts of milk and whole egg. Protein efficiency ratios for 4-week periods for the unbaked ingredients were: baking-powder biscuits, 2.4; muffins, 2.9; griddlecakes, 3.6; and 4.2 for a cookie mix over a 3-week period. The ratios were about 15% lower when the mixtures were baked.

Milk and eggs exerted a considerable supplementary effect, since the determined protein efficiency ratios were more than 60% higher than those calculated from the individual ingredients. White flour is deficient in isoleucine, lysine, methionine and cystine, threonine, tryptophan, and valine when compared with the F. A. O. reference protein. With 76% of the protein from white flour and 24% from milk, calculations showed that isoleucine, threonine, and valine were no longer limiting. Increasing amounts of milk and egg protein increased the protein efficiency ratios. When 52% of the protein was supplied by white flour and the rest by milk and eggs, as in griddlecakes, the protein efficiency ratio was nearly as high as was that for whole egg. Comparison with the reference protein showed this mixture of white flour, milk, and egg protein to be still somewhat low in methionine and cystine and in tryptophan.

Many investigators have studied the quality of the protein in wheat flour, bread, and cereals. Much of the work has been concerned either with improvement of bread and flour by the addition of amino acids or of natural foods such as milk solids and soybean flour, or with the effect of heat on destruction of protein quality. Owing to the supplementary value of the milk and egg, the protein in such products as breads, cakes, and cookies (which are often thought of as starchy) may be of fairly high quality. Because the quality of the protein has been underrated in these foods, protein efficiency ratios were determined for representative baked products containing various proportions of milk, egg, and flour protein. Losses in protein quality due to baking were also determined.

Materials and Methods

Baked Products. Baking-powder biscuits, muffins, and griddlecakes were prepared from standard recipes according to the formulas given in Table I. The cookie formula was designed to give a higher percentage and a better quality of protein than is usually found in this

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product. Baking times and temperatures were: biscuits, 12 minutes at 425°F. (218°C.); muffins, 23 minutes at 400°F. (204°C.); griddlecakes, 2 minutes at 400°F. (204°C.); and cookies, 10 minutes at 350°F. (177°C.).

The composition of the products on both fresh and air-dried bases is shown in Table II. The factors used to convert nitrogen to protein in the baked products, with the exception of the cookies, were calculated from the percent milk, egg, and flour protein in each product, using 5.70 for all-purpose flour, 6.38 for milk, and 6.25 for whole egg.

Diets. To study the protein quality before and after baking, both the unbaked ingredients and the air-dried baked products were fed to rats. In addition, ingredients containing protein — all-purpose flour, "instant" nonfat dry milk, and dried whole egg — were tested separately.

For the unbaked controls, the dry ingredients were thoroughly mixed. Shortening was added when the diets were made. The baked products were broken into small pieces, air-dried at room temperature

TABLE I

INGREDIENTS IN BAKED PRODUCTS

INGREDIENT	PARTS BY WEIGHT				
	Baking-Powder Biscuits	Muffins	Griddlecakes	Cookies	
Flour, all-purpose	100.0	100.0	100.0	73.5[a]	100.0
Wheat germ	....	....	....	26.4	
Nonfat dry milk, instant	8.8	10.6	18.5	50.0	
Hydrogenated shortening	25.8	14.6	28.2	69.7[b]	
Dried whole egg	....	6.0	6.0	18.7	
Salt	1.4	1.4	1.4	3.6	
Baking powder	4.3	3.5	4.3	5.6	
Sucrose	....	11.4	....	111.0	

[a] Whole-wheat flour.
[b] Margarine.

TABLE II

COMPOSITION OF FRESH AND AIR-DRIED BAKED PRODUCTS

PRODUCT	MOISTURE	NITROGEN	PROTEIN CONVERSION FACTOR	PROTEIN
	%	%		%
Baking-powder biscuits				
Fresh	29.0	1.30	5.86	7.6
Air-dried	3.8	1.76	5.86	10.3
Muffins: fresh	41.2	1.27	5.94	7.5
Air-dried	6.6	2.02	5.94	12.0
Griddlecakes: fresh	51.0	1.12	6.17	6.9
Air-dried	6.0	2.14	6.17	13.2
Cookies: fresh	14.7	1.89	6.25	11.8
Air-dried	7.4	2.05	6.25	12.8

in circulating air, and finely ground.

The diets contained 1.60 ± 0.1% nitrogen; 2% Hubbel, Mendel, and Wakeman salt mixture[2]; 10% hydrogenated vegetable fat in experiments 2 and 4, and 13 to 19% in experiments 1 and 3; and sucrose. Diets made from flour, unbaked ingredients, or air-dried baked product contained from 55 to 96% of the protein source. The milk diet in experiment 2 contained 10% fat, 30% dry milk, and 58% sucrose; in experiment 3, 13% fat, 29% dry milk, and 56% sucrose. The egg diet for the 4-week period contained 24% dry whole egg, 74% sucrose, and no added fat; for the 3-week period, 21% dry whole egg, 67% sucrose, and 10% hydrogenated vegetable fat.

All animals were fed a supplement of B vitamins in 20% ethanol solution, and vitamins A, D, and E in cottonseed oil[3].

Feeding and Experimental Procedure. The selection and care of the rats was essentially the same as in a previous study on bread (7). Weanling rats, 21 days old, were used. For 3 days before the experiment began, the animals were fed a starter diet containing 6% casein, 10% cottonseed oil, 4% USP XII salt mixture, and 80% sucrose, plus the vitamin supplements. They were then divided into groups of 10 animals each, similar in average weight, number of males and females, and litter representation. Experimental diets were fed *ad libitum* for 4 weeks.

Results

Food intakes, weight gains, and nitrogen and protein efficiency ratios for animals on the different diets are shown in Table III. The greatest weight gains were found in the animals on the unbaked diets, with the cookie diet giving the largest gain, followed by griddlecakes, muffins, and baking-powder biscuits, in that order. Weight gains of animals fed on the unbaked cookie diet for a 3-week period were a little greater than those of animals fed whole egg over the same period, and were larger than those of the group fed on milk for four weeks. Animals fed the unbaked griddlecake diet gained as much as those fed on milk. Animals fed the baked diets gained less than those fed on the unbaked diets, with the exception of biscuits. Groups fed on the baked and the unbaked biscuit diets had the same average weight gains.

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[2] Grams per kilogram of salt mixture: $CaCO_3$, 543.0; $MgCO_3$, 25.0; $MgSO_4$, 16.0; NaCl, 69.0; KCl, 112.0; KH_2PO_4, 212.0; $FePO_4 \cdot 4H_2O$, 20.5; KI, 0.08; $MnSO_4$, 0.35; NaF, 1.00; $Al_2(SO_4)_3K_2SO_4$, 0.17; $CuSO_4$, 0.90.

[3] Amounts received per animal per day: thiamine HCl, 43 γ; riboflavin, 43 γ; niacin, 172 γ; biotin, 8.6 γ; folic acid, 8.6 γ; menadione, 30 γ; vitamin B_{12}, 206 γ; pyridoxine HCl, 61 γ; calcium pantothenate, 172 γ; choline, 5 mg.; vitamin A, 100 I.U.; vitamin D, 10 I.U.; alpha tocopherol, 0.5 mg.

TABLE III

PROTEIN QUALITY OF INGREDIENTS AND OF BAKED PRODUCTS FED TO RATS[a]

Exp. No.	Protein Source	Total Weight Gain	Total Food Intake	Total Nitrogen Intake	Nitrogen Efficiency Ratio[b]	Protein Efficiency[c]			
						Ratio	St. dev.	Loss with Baking	"t" Value
		g	g	g				%	
1	Cookies, unbaked[d]	112	255	4.30	26.0	4.17	0.22	...	
	Cookies, baked[d]	74	198	3.31	22.0	3.52	0.16	15.5	6.58**
2	Nonfat dry milk, instant	102	297	4.94	20.6	3.22	0.24	...	
	Dried whole egg	120	303	5.15	23.3	3.72	0.29	...	
	Muffins, unbaked	80	286	4.46	17.5	2.94	0.34	...	
	Muffins, baked	64	285	4.26	15.0	2.52	0.14	14.5	3.69**
3	Nonfat dry milk, instant	105	285	4.74	22.1	3.46	0.15	...	
	Dried whole egg[e]	107	236	3.90	27.5	4.46	0.21	...	
	Baking-powder biscuits, unbaked	40	202	2.86	13.8	2.36	0.18	...	
	Baking-powder biscuits, baked	40	196	3.31	12.1	2.06	0.21	12.7	3.45**
	Griddlecakes, unbaked	102	307	4.70	21.5	3.58	0.23	...	
	Griddlecakes, baked	81	277	4.56	17.7	2.94	0.19	17.8	6.48**
4	All-purpose flour[f]	12	157	2.58	4.8	0.85	0.22	...	

[a] Experimental period 4 weeks, 10 animals per group. Diets contained 1.60 ± 0.1% N, as-is moisture basis.
[b] Weight gain per g. of nitrogen eaten.
[c] Weight gain per g. of protein eaten.
[d] Experimental period 20 days.
[e] Experimental period 21 days.
[f] Eight animals per group.

Nitrogen and protein efficiency ratios for the unbaked diets fell in descending order as follows: the cookie diet, griddlecakes, muffins, and baking-powder biscuits – the same order as shown by weight gains. Protein efficiency ratios for the unbaked ingredients were significantly greater than for the baked products, as is shown in Table III. The loss on baking ranged from 13 to 18%, which is within the range found in previous work on bread (7).

The difference in protein efficiency ratios between unbaked griddlecakes and egg, both for 4 weeks, was not statistically significant by the "t" test. The efficiency ratios for the egg diet for a 3-week period were only slightly greater than those for the unbaked cookies for 3 weeks, the "t" value for the differences between the protein efficiency ratios of these two groups being 3.02, which is significant at the 2% level. Some of the animals on the egg diet had diarrhea. Because this diet contained a fairly large amount of sucrose whereas the cereal diets had relatively little, the effect of the difference in carbohydrate was tested. Three groups of animals were fed diets containing 21% dried whole egg for a 3-week period. In addition to the salt mixture the diets contained 1) 77% sucrose, 2) 77% corn starch, and 3) 67% sucrose and 10% hydrogenated vegetable fat. The data for the third group are given in Table III. Animals of the first two groups ate 248 and 283 g. of food, gained 108 and 116 g. of weight, and had protein efficiency ratios of 4.22 ± 0.22 and 3.95 ± 0.24, respectively. The difference in protein efficiency ratios between these two groups was significant at the 5% level. There was no diarrhea in the animals on corn starch. The cookies were tested as a preliminary study and it is unfortunate that the egg diet was not tested simultaneously. The egg diet containing 10% added fat was used for comparison with the cookie diet because the fat contents were similar. The protein efficiency ratio of the cookie diet was similar to the values for the egg diets whether

TABLE IV

COMPOSITION OF FRESH BAKED PRODUCTS

Product	Content per Average Serving					Protein Efficiency Ratio[b]	Protein Calories
	Weight	Moisture	Fat[a]	Protein	Calories[a]		
	g	g	g	g			% of total
Baking-powder biscuits	20	5.8	3.0	1.5	70	2.4	8.7
Muffins	38	15.7	3.0	2.9	101	2.5	11.4
Griddlecakes	40	20.4	4.4	2.8	98	2.9	11.3
Cookies	24	3.5	4.8	2.8	102	3.5[c]	11.1

[a] Calculated.
[b] Gain in weight per g. of protein eaten, 10% protein in diet, 4-week period.
[c] Three-week period.

sucrose or corn starch was present.

The composition of average servings of fresh baked products is given in Table IV.

Discussion

Quality of the Protein. The supplementary effect of one protein on another, whereby the quality of a mixture of two proteins is better than the average of the two fed singly, has been known since the classic work of Osborne and Mendel. An example of this effect, when milk and eggs were incorporated into wheat products, is shown in

TABLE V

SUPPLEMENTAL VALUE OF MILK AND EGG PROTEIN IN WHEAT PRODUCTS FED TO RATS

Product, Unbaked Ingredients	Source of Protein			Protein Efficiency Ratio		
	Flour	Milk	Eggs	Calculated[a]	Determined 4 Weeks	Increase
	%	%	%			%
White flour	100.0	....	....	...	0.85	..
Milk	....	100.0	....	...	3.22	..
					3.46	
Whole egg	....	....	100.0	...	3.72	..
					4.46[b]	
Baking-powder biscuits	76.0	24.0	....	1.47	2.36	64
Muffins	61.8	22.5	15.7	1.83	2.94	61
Griddlecakes	52.1	34.6	13.3	2.13	3.58	68
Cookies	18.2[c]	44.0	21.6	3.0	4.17[b]	..
	16.5[d]					

[a] Weighted mean of the protein efficiency ratios of the individual ingredients.
[b] Three-week period.
[c] Whole-wheat flour, protein efficiency ratio of 1.5 used for calculation.
[d] Wheat germ, protein efficiency ratio of 2.9 used for calculation.

Table V. The determined protein efficiency values before baking were more than 60% higher and after baking 39% higher than the values calculated from the individual proteins. In other words, if the protein efficiency ratios were merely additive, the ratio for white flour would need to be 2.1 in the case of baking powder biscuits, 2.6 for muffins, and 3.5 for griddlecakes, rather than 0.85, to obtain the determined values.

It is also well known that wheat, especially in the form of refined flour, is deficient in some of the essential amino acids. When the data of Orr and Watt (5) for the amino acid composition of white flour are compared with the amino acid content of the F.A.O. reference protein (3), flour is shown to be low in lysine, methionine and cystine, valine, tryptophan, threonine, and isoleucine (Table VI). Egg protein has no deficiency, whereas milk is low in methionine and cystine.

TABLE VI

COMPARISON OF AMINO ACID CONTENT OF PROTEIN IN INGREDIENTS AND IN BAKED PRODUCTS

Product, Unbaked Ingredients	Amino Acid[a]					
	Isoleucine	Lysine	Methionine and Cystine	Threonine	Trypto-phan	Valine
	mg/g N	*mg/g N*	*mg/g N*	*mg/g N*	*mg/g N*	*mg/g N*
F.A.O. reference protein (3)	270	270	270	180	90	270
White flour	*262*	*130*	*189*	*164*	*70*	*246*
Milk	407	496	*213*	294	90	438
Whole egg	415	400	342	311	103	464
Baking-powder biscuits	294	*210*	*194*	193	*75*	288
Muffins	315	*247*	*217*	212	*79*	319
Griddlecakes	329	283	*216*	225	*80*	336
Cookies	357	390	*232*	279	*85*	392

[a] Calculated from data by Orr and Watt (5).

When 26% of the flour protein was replaced by milk, as in baking-powder biscuits, calculation of the amino acid composition showed that the mixture was no longer low in isoleucine, threonine, and valine, but was still low in lysine, tryptophan, and methionine and cystine. With increasing amounts of milk and egg (griddlecakes and cookies), the lysine became adequate. Tryptophan and methionine and cystine increased, but did not reach the level of the reference protein.

The protein efficiency ratios for the unbaked ingredients of griddlecakes and cookies compared favorably with those of egg protein, even though none of the essential amino acids, with the exception of tyrosine in the cookies, was equal to the level of these amino acids in egg protein. Except for tryptophan and methionine and cystine, however, the essential amino acid composition of the protein in these products did reach the levels set for the F.A.O. reference protein. Whether or not the quality of the protein in the griddlecake and cookie ingredients is really nearly equal to that of egg protein should be checked by other methods of protein quality evaluation, such as the amount of carcass nitrogen deposited.

Judged by the foregoing results, other flour products containing eggs and milk would be expected to have fairly high-quality protein. Yellow cake, containing about 8% protein in the fresh product, derives 40 to 50% of its protein from cake flour, about 40% from whole egg, and from 10 to 20% from milk. The quality of the protein might well be as good as, or better than, that found in griddlecakes, since the proportion of egg protein is higher.

Effect of Baking. The protein efficiency ratios of the baked prod-

ucts were consistently lower than those of the unbaked ingredients. Although no systematic study was carried out on the effect of time of baking, temperature of baking, amount of browning, or surface area, no clear-cut relationships were noted between these factors and the percentage loss of protein quality with baking. Griddlecakes, with noticeable browning, baked for 2 minutes at 204°C., decreased 17.8% in protein efficiency ratio; cookies, with no evidence of browning on the top surface and only a slight amount of browning on the undersurface, baked for 10 minutes at 177°C., decreased 15.5%; while muffins, baked for 23 minutes at 204°C., and with lightly browned crusts, decreased 14.5%.

Loss of protein quality as a result of baking has previously been observed in this laboratory in wheat bread (7) and in rye bread (8). Although baking times and temperatures for the breads were similar (30–35 minutes at 204°–221°C.), rye bread showed less baking loss, 9–12%, than did wheat bread, 11–33% (average 21%).

Decrease in protein quality with baking has been noted by several workers, and has usually been attributed to the formation of crust. Factors contributing to the poorer nutritive value of the crust are the decreased digestibility and the destruction of lysine (4). Block *et al.* (1) observed that the protein efficiency ratio of a high-protein cake mix decreased from 3.3 to 2.4 (27%) on baking at approximately 200°C. for 15 to 20 minutes. Rosenberg and Rohdenburg (6) found the loss of lysine in bread due to baking to be about 11% by microbiological assay, with losses varying from 2.4 to 15.8%. Egli *et al.* in 1957 (2) noted that it is difficult to prevent the deterioration of amino acids when biscuits are baked in an oven. In their experiment, biscuits (23% protein) were digested *in vitro* with pepsin and pancreatin, and the liberation of amino nitrogen, tryptophan, methionine, and lysine was determined in the biscuits and compared with the values obtained from the uncooked mixture. They found that tryptophan, methionine, and lysine deteriorated about 20% if the biscuits were baked *à point*, and about 50% if they were lightly browned. The deterioration was more pronounced at 170°C. than at 140°C.; it increased with the length of baking and decreased with the thickness of the biscuits.

The change in the quality of the protein of baked products is complex, and probably involves factors other than the crust. Work in our laboratory[4] has shown that the quality of the protein may decrease although no brown crust is formed, for example in bread baked in microwave oven, or that the quality may be increased even in

[4] Kennedy, Barbara M. Unpublished observations (1959).

the presence of visible browning under other conditions of cooking.

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A POLARIMETRIC STUDY OF FLOUR DIASTATIC VALUE[1]

A. H. Woodhead[2] and Catherine A. Wyatt[2]

ABSTRACT

The diastatic values of flour samples measured polarimetrically differ in a regular and notably simple manner from results obtained by chemical reduction methods of analysis. The reducing substances present in bran and germ were not recorded by the polarimeter, and the results by the latter method are therefore suggested as affording a more convenient measure of the main products of flour autolysis.

There are several well-known procedures for estimation of flour diastatic values, the one currently used being that of Blish and Sandstedt (1), which depends upon a chemical reduction principle. Molin (5) has described a refractometric method to determine the total products of starch hydrolysis as an estimation of germination damage in wheat, by autolysis at 62°C. Munz and Bailey (7) extended this work

[1] Manuscript received June 17, 1959.
[2] Unga Limited, Nairobi Mills, Nairobi, Kenya.

by including in their refractometric studies the effects of temperature and of various buffer solutions, and the incremental effects of added malt preparations.

Kent-Jones and Amos (3) used the polarimeter in studying the dextrinogenic activity of oat flour, using autolysis at 62°C. and basing their calculations upon the large specific optical rotatory power for dextrins which they assumed to be +200°. Though the method was not applied to wheat flour, they claimed that it sufficed to put a number of oat flour samples in the correct order of alpha-amylase activity as indicated by the method of Hills and Bailey (2).

The experimental work described in this paper concerns the estimation of what are commonly referred to as "maltose values" by autolysis of flour suspensions at 30°C. in a weak buffer solution, the result being determined by polarimetric measurements on the clear extract obtained by a technique developed by the present authors. The accurate measurement of optical rotation depends upon the use of perfectly clear solutions; hence it was necessary to develop clarifying agents of high efficiency. These were found to act conveniently as diastasis inhibitors, thereby making the procedure experimentally stable and reliable.

Theory

The simplified expression for specific optical rotation at 20°C. referred to sodium (D) light is:

$$[a]_D^{20} = 100R/C.l$$

where R is the measured angular rotation in degrees;

C is the concentration of substance in 100 ml. solvent; and

l is the length of the tube used, in decimeters.

This expression is applicable to dilute solutions having unit density.

The high specific rotation for maltose (+138°) suggests that a reasonably large rotation could be anticipated at a maltose concentration of the order of 1.5% using a 2-dm. tube. By using 25-g. flour samples and a total of 69 ml. of liquid diluents, including clarifying and inhibiting reagents, it is readily seen that R = percent maltose, numerically. This assumes, for the purpose of experimental design only, that maltose is the sole product of diastasis.

It is known that flour contains small amounts of natural sugars (4,6,8,9) which have been characterized. It was expected that the effect of these sugars would be small but would be superimposed upon the final results, assuming them to have a definite resultant optical rotation. It was decided to compare the polarimetric results with those

obtained by the Blish-Sandstedt procedure on the same samples.

Materials and Methods

Materials. The flour samples used included straight-run bakers' and family white flours of 75% extraction or lower, whole-meal, brown or "standard" flour of 85% extraction, biscuit and mill stream flours of very low diastatic value, and medium wheatmeals known in East Africa as "Atta" and made for the local Indian community. These provided a wide range of diastatic activity.

The reagents used were:
Acetic acid/sodium acetate buffer, pH 4.6–4.8, 0.05M;
Potassium ferrocyanide solution, 15% w/v;
Zinc acetate, 15% w/v, in 50% v/v acetic acid.

The equipment used included a simple polarimeter fitted for sodium (D) light; a centrifuge accepting 100-ml. tubes; flat-bottomed, wide-necked flasks of 250-ml. capacity; a thermostatically controlled water bath operating at 30°C. ± 0.1°C.; and fine filters either of paper (Whatman No. 42, 12.5-cm.) or sintered-glass Büchner funnels of No. 4 porosity and 60-ml. capacity.

Method of Analysis. A storage flask containing sufficient buffer solution was kept in the 30°C. bath. Flour samples of 25 g. ± 0.01 g. were weighed on paper and transferred to the flat-bottomed flasks, together with about 10 g. of coarse sand. The flour and sand were mixed by rotation of the flasks, and then conditioned in the bath for 15 minutes. The flasks were then removed; 55 ml. of buffer solution at 30°C. were quickly added to each; the mixture was homogenized by a vigorous swirling motion and replaced in the bath. The samples were incubated for 1 hour, the pH being 5.0. After 1 hour the flasks were removed, and 7 ml. of the potassium ferrocyanide solution were added to each and mixed by shaking; the flasks then were placed in a water bath at room temperature. To each were added 7 ml. of the zinc acetate/acetic acid solution, with thorough shaking. The pH fell to 4.0. The mixtures were then centrifuged for 15 minutes at 2,500 r.p.m. at a mean radius of 15 cm.

The resulting supernatant liquid was filtered without loss through Whatman No. 42 (12.5-cm. diameter) papers or through the sintered-glass funnels under moderate suction. The whole of the liquid was filtered by one of these methods and the perfectly clear filtrate was polarized in a 2-dm. tube using sodium (D) light. Results reproducible to ± 0.2° were afforded by this procedure. Mutarotation was not observed, and measurements may therefore be made immediately or at convenience. Solutions in fact had prolonged stability and could

be checked either immediately or on the following day by the same observer or by an independent one.

Results and Discussion

A comparison of the polarimetric values with those obtained by the Blish-Sandstedt procedure is shown on Fig. 1. Linear relationships were

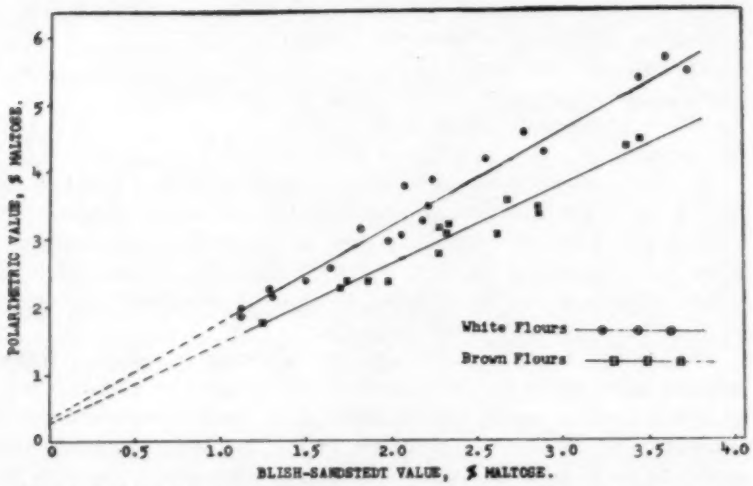

Fig. 1. Relationship between polarimetric and Blish-Sandstedt diastatic values. Circles indicate white flours; squares indicate brown flours.

found, the regression equation being slightly but positively different for white flours as compared with that for the brown flours. The extrapolated curves almost converge on the ordinate axis representing the polarimetric values. The polarimetric values are always higher than the chemical values, both being expressed as a percentage of maltose in the flour. The results show that polarimetric value equals:

For white flours ..... (B-S Value) $1.438 + 0.33$ $r = 0.98$
For brown flours ..... (B-S Value) $1.19 + 0.23$ $r = 0.97$

The correlation coefficients are highly significant. The constants 0.33 and 0.23 respectively indicate points of zero diastasis; i.e., they are the polarimetric equivalents of natural nonreducing sugars initially present in the two types of flour. At the dilution used, they correspond to approximately 0.8% sucrose or its optical rotatory equivalent of a mixture of nonreducing sugars. Detailed independent

work on these natural sugars by Williams and Bevenue (9), by Koch, Geddes, and Smith (4), and by Montgomery and Smith (6) supports this result. Their collective findings may be summed up in the statement that wheat flour contains 0.8–1.1% of nonreducing sugars, and only 0.1% reducing sugars.

The regular divergence between the chemical and the optical assays of the products of diastasis for both types of flour is somewhat larger than one would expect. The reducing value of maltose has been carefully evaluated in the work of Blish and Sandstedt, while the specific optical rotation of pure maltose is an accepted physical constant. It is likely that much if not all of this divergence is due to the presence of dextrin or dextrin-maltose complexes formed by autolysis. These are known to have a higher specific optical rotation than pure maltose, according to Kent-Jones and Amos.

On the other hand, bran extracts have a very high chemical reducing value and practically no optical activity – points which we have checked experimentally. These features of bran account for the small but apparently significant difference in the regression equations for the brown and white flours. It is suggested that the polarimetric scale is more convenient than the chemical reduction values given by the Blish-Sandstedt procedure, since the reducing substances other than maltose which are present in bran and germ are not measured by the polarimeter, the latter recording only the optically active products of autolysis, which are chiefly maltose and the malto-dextrins.

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CHANGES IN THE SOLUBLE CARBOHYDRATES DURING BROWNING OF WHEAT EMBRYOS[1]

PEKKA LINKO, YU-YEN CHENG, AND MAX MILNER[2]

ABSTRACT

Changes in the soluble carbohydrates of wheat embryos as affected by storage at temperature and moisture values pertinent to conditions which produce germ-damaged ("sick") wheat in commercial grain were investigated chromatographically. From the total sugar content of 28.6% in commercial wheat germ, raffinose (38.1%) and sucrose (55.9%) are the major components, with fructose (2.8%), glucose (2.1%), and melibiose (1.1%) present in smaller quantities. Storage for 8 days at various moisture contents from 8.9 to 25%, and temperatures from 29° to 50°C., produced characteristic increases of reducing sugars, at the expense of the nonreducing.

Browning, as indicated by fluorescence, increased with moisture content and temperature, but only after discoloration was visually apparent, and particularly at moisture values beyond 15%. A decrease in nonreducing sugars, as well as an increase in reducing carbohydrates which apparently form browning intermediates with available free amino acids, preceded marked increases in fluorescence.

At moisture levels related to practical storage conditions, several unknown sugarlike compounds appeared quickly in stored commercial germ. These compounds disappeared later as fluorescence values rose. However, only one of these unknowns appears in intact viable wheat grains when sufficient moisture permits germination.

It has been shown recently that only moderate wetting of wheat embryos, either excised or in the intact kernel, immediately activates several enzyme systems that cause rapid changes in the amounts of free amino acids (12,13). This led to the suggestion that amino acids liberated from proteins might, under practical storage conditions, react nonenzymatically with reducing sugars in the embryo to yield the browning reaction products shown to be characteristic of "sick" wheat (4). For these reasons, the behavior of soluble carbohydrates during storage of wheat at different moistures and temperatures becomes important in formation of "sick" wheat.

The discovery by Richardson and Crampton (18) of sucrose as the predominant soluble carbohydrate in wheat germ was soon followed by the crystallization and identification of raffinose (19). Liebig (11) published evidence for the presence of glucose in wheat meal. Kluyver (9) determined, both by chemical methods and by fermentation tech-

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[2] Respectively, Assistant Professor, Graduate Research Assistant, and Professor, Kansas State University.

niques, the presence of fructose, glucose, raffinose, and sucrose in ungerminated wheat. Dubois (5) crystallized raffinose and sucrose from wheat germ and also obtained chromatographic evidence for the presence of trace amounts of fructose and glucose.[3] The evidence (10,20) for the presence of fructose, glucose, maltose, and sucrose in wheat flour was confirmed by Montgomery and Smith (15) through isolation of the crystalline compounds.

Ramstad and Geddes (17) found a marked increase in reducing sugars in soybeans stored at more than 15% moisture content. An equally marked decrease in nonreducing sugars followed. According to Milner *et al.* (14), total sugars in wheat decrease with storage at moisture levels above 15.4%; the reducing sugars showed little or no increase below 20.8% moisture. Montgomery and Smith (16) pointed out that the quantity of soluble carbohydrates in wheat is likely to depend upon the highest moisture level to which the grain has been exposed. Houston *et al.* (8) have reported similar changes in rice, where a decrease in nonreducing sugars is followed by some increase in reducing sugars at moisture values above 14%. They found that at 32°C. and 16.5% moisture, nearly all nonreducing sugars were lost. Bottomley *et al.* (2) have shown that decrease in nonreducing sugars in corn parallels mold count more closely than does increase in fat acidity.

The present work was undertaken to study the changes in individual soluble carbohydrates in commercial wheat germ during short storage periods under various conditions of moisture and temperature.

Materials and Methods

Materials. Fresh granular wheat germ containing 8.9% moisture, supplied by International Milling Co., Minneapolis, Minn., was used. The germ was stored in a moisture-proof container at +4°C.

For germination experiments, an Ohio-grown soft red winter wheat, variety Seneca, with moisture content of 11.6% was used.

Moisture. Moisture content (wet-weight basis) was determined by drying the samples for 1 hour at 130°C. Samples of different moisture contents were prepared by adding the calculated amount of distilled water, followed by vigorous mixing. Samples were stored in moisture-proof containers at various temperatures, and remained mold-free during the experimental periods used.

Determination of Sugars. Soluble carbohydrates were extracted from wheat germ with 70% (w/v) ethanol, after which the extract

[3] These studies are presented in detail in the paper by Dubois, Geddes, and Smith entitled "The Carbohydrates of the Gramineae. X. A Quantitative Study of the Carbohydrates of Wheat Germ" which appears in this issue (pp. 557-560). W. F. G., *Editor.*

was passed through Amberlite IR-120 (H^+) resin. The eluate containing the sugars was distilled in vacuum to a small volume.

Whatman No. 4 filter paper was used without prewashing for chromatography. The first solvent for two-dimensional chromatography was water-saturated phenol (Merck, reagent grade); the second, 1-butanol-acetic acid-water (63:10:27, by volume). The latter also was used as a solvent for one-dimensional chromatography. The chromatograms were normally developed at 27°C. for about 36 hours in 1-butanol-acetic acid-water, at which time fructose migrated to the bottom edge of the paper. However, for the detection of possible presence of pentoses and tetroses, shorter developing times were used. Eight to ten hours' development in phenol-water was found satisfactory.

Two principal reagents were used to detect sugars on paper chromatograms:

1. *Silver nitrate.* This reagent was used for general detection of sugars. The chromatogram was dipped into silver nitrate solution (0.1 ml. of saturated aqueous silver nitrate diluted to 20 ml. with acetone, after which silver nitrate was brought back to solution by addition of water droplets), dried at room temperature, and then dipped into 0.5*N* sodium hydroxide in aqueous methanol.[4] Black spots developed immediately. Chromatograms were again dried at room temperature for 5 to 10 minutes, after which they were slowly pulled through Kodak X-ray fixing solution (1). Chromatograms were finally thoroughly washed with tapwater to remove excess fixing solution. Paper tearing could be avoided by careful handling. The method yielded relatively stable chromatograms showing black spots against white background. The individual sugars were identified by co-chromatography with known carbohydrates.

2. *2,3,5-Triphenyltetrazolium chloride.* Chromatograms were dipped into a freshly prepared mixture of equal volumes of 4% methanolic 2,3,5-triphenyltetrazolium chloride and 1*N* sodium hydroxide in aqueous methanol (6). The papers were then heated 10 minutes at 75°C. in a water-saturated atmosphere. Red spots developed on a slightly pink background. This reagent is much less sensitive than silver nitrate, and it was used only to detect reducing sugars. For quantitative determinations, the size of the largest spot was used as a guide in cutting out all the other sugar spots, as well as a blank. The color was extracted with 5 to 10 ml. of methanol-acetic acid mixture (10:1, v/v), and the extinction was read at 482 mμ.

[4] Sodium hydroxide was dissolved in a minimum amount of distilled water, and diluted to desired volume with anhydrous methanol.

One-dimensional runs in 1-butanol-acetic acid-water were satisfactory for the determination of fructose, glucose, maltose, and melibiose. Raffinose and sucrose were determined according to the method of Williams and Bevenue (21) by hydrolyzing the solution to be analyzed on the origin of a chromatogram by means of invertase. Two applications of 10-μl. spots each of invertase (National Biochemicals Corp., 200 mg. ml.) per 2-μl. spot of sugar solution were made, after which fructose, glucose, and melibiose were determined as usual.

Results and Discussion

As indicated by Table I, raffinose and sucrose are the major soluble carbohydrates in commercial wheat germ; fructose, glucose, and melibiose are present in smaller quantities. Other unidentified sugars are

TABLE I
SOLUBLE CARBOHYDRATES IN COMMERCIAL WHEAT GERM
(Moisture-free basis)

SUGAR	QUANTITY	PERCENT OF TOTAL SUGAR
	mg/g	%
Fructose	8	2.8
Glucose	6	2.1
Melibiose	3	1.1
Raffinose	109	38.1
Sucrose	160	55.9
Other	Traces	
Total	286	100.0

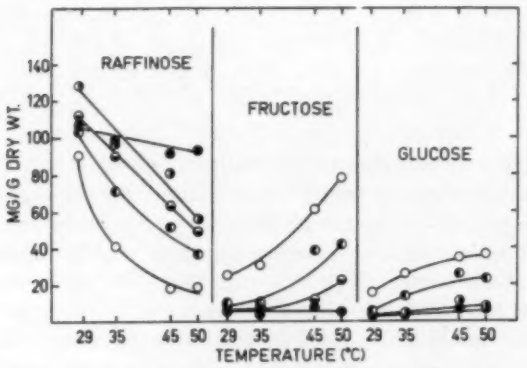

Fig. 1. Effect of temperature on the change in concentration (moisture-free basis) of raffinose, fructose, and glucose in commercial wheat germ stored 8 days at various moisture levels. Percent moisture: ● = 8.9; ◑ = 13; ◒ = 15; ◐ = 18; ○ = 25.

present only in traces. The total amount of soluble sugars found in the present work is close to 29% (moisture-free basis), a somewhat higher value than Dubois (5,16) reported (see footnote 3).

Figure 1 shows the effect of temperature on the concentration of fructose, glucose, and raffinose after small samples of commercial wheat germ were stored at different moisture contents and at various temperatures for 8 days. If germ is kept at the original 8.9% moisture level, temperature increase up to 50°C. causes almost no changes in sugar concentrations. Changes become more pronounced, however, with increasing moisture content. Raffinose decreases, and reducing sugars increase with increasing temperature. Figure 2 shows in greater

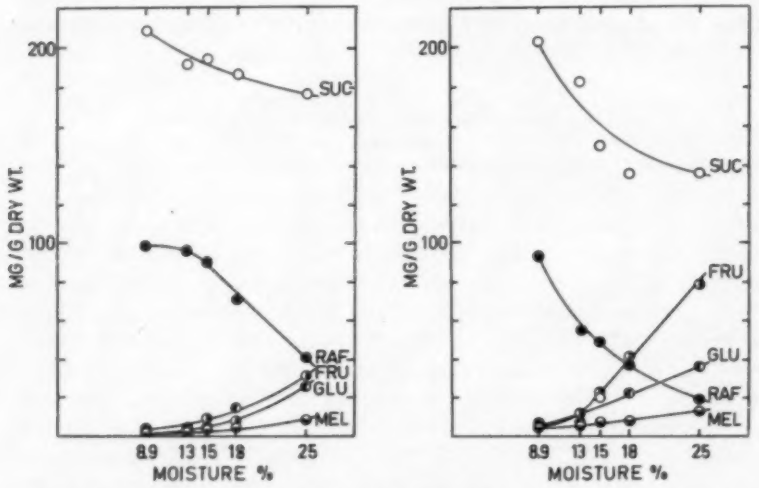

Fig. 2. Effect of moisture content on the change in the amounts (moisture-free basis) of sugars in commercial wheat germ stored 8 days at 35°C. (left), and 50°C. (right).

detail the effect of moisture content at 35° and 50°C. on the concentrations of soluble sugars, indicating that nonreducing sugars decrease and reducing sugars increase with increasing moisture content.

Table II shows the degree of browning of these samples as measured by the fluorescence (3,4). Browning increases when moisture and temperature increase. The primary browning reaction products are not, however, fluorescent; visually detectable browning of the germ occurs noticeably before any significant increase in fluorescence value. A marked increase in fluorescence appears only at moisture levels above 15%. In general, decrease in nonreducing sugars is followed by

TABLE II

EFFECT OF TEMPERATURE AND MOISTURE ON DEVELOPMENT OF FLUORESCENCE IN WHEAT GERM DURING 8 DAYS OF STORAGE

(911 mg.: moisture-free basis)

H_2O	Fluorescence Units			
	29°C.	35°C.	45°C.	50°C.
%				
8.9	4	3	6	7
13	4	4	10	15
15	4	4	19	66
18	65	79	94	6,800
25	92	3,200	5,000	17,000

increases in browning. Increases in glucose and fructose are not so great as might be expected from the breakdown of sucrose and raffinose alone. This is most likely due to the reaction of reducing sugars with free amino acids to form the intermediates of nonenzymatic browning.

As shown in Fig. 3, commercial wheat germ contains, in addition to the above-mentioned sugars, three other sugarlike compounds, one of which coincides with lactose. While the chromatographic evidence for the possible presence of lactose in wheat germ is only presumptive, work is in progress to isolate this compound in crystalline form. The two other compounds are marked as spots 100 and 101. During 8 days' storage at 8.9% moisture, two additional unknown compounds, 102 and 103, appear. When the moisture content increases, compound 101 rapidly disappears, whereas compounds 100, 102, and 103 increase very markedly. A small amount of galactose also appears at higher moisture levels. Glass and Geddes (7) recently isolated D-galactose, as well as D-glucose, D-fructose, glycerol, and myo-inositol, from wheat which had been stored under nitrogen at 16 to 18% moisture for 24 weeks at 30°C. Compounds 102 and 103 reach a maximum at 15% moisture level, after which they begin to decrease. On the other hand, several additional unknown spots appear on chromatograms above this moisture level. Because fluorescence also has a sharp increase at this point, it is likely that some of the unknowns may be labile primary intermediates in the nonenzymatic browning reaction. The position of the above-mentioned unknowns on the chromatogram, with the exception of the "lactose" spot, does not coincide with any of the known simple carbohydrates. Work is in progress to establish their identity.

For comparison, changes in soluble carbohydrates during germination of intact wheat grains were also studied. Figure 4 shows that fruc-

tose, glucose, and maltose are the major soluble carbohydrates in the intact wheat grains. Raffinose disappears at an early stage of germination, whereas fructose, glucose, and maltose greatly increase. After a few days, a series of oligosaccharides appear at the upper right hand corner of the chromatograms. Compound 100 was the only one of the

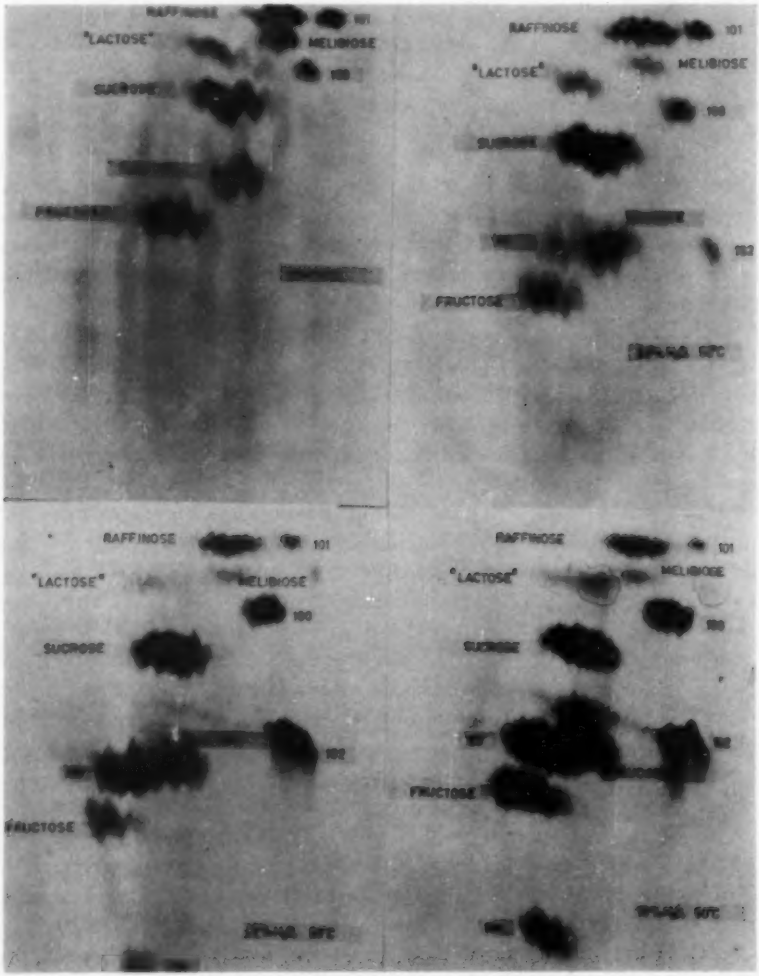

Fig. 3. Two-dimensional paper chromatograms of soluble carbohydrates in commercial wheat germ stored 8 days at 50°C. and at various moisture levels. Amounts used for each chromatogram correspond to 5 mg. germ (moisture-free basis). Spots are identified with silver nitrate. (See also continuation, opposite page.)

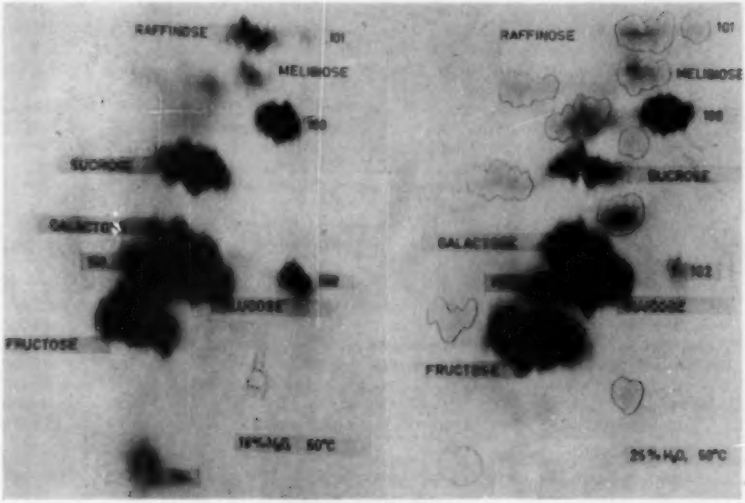

Fig. 3 (Continuation).

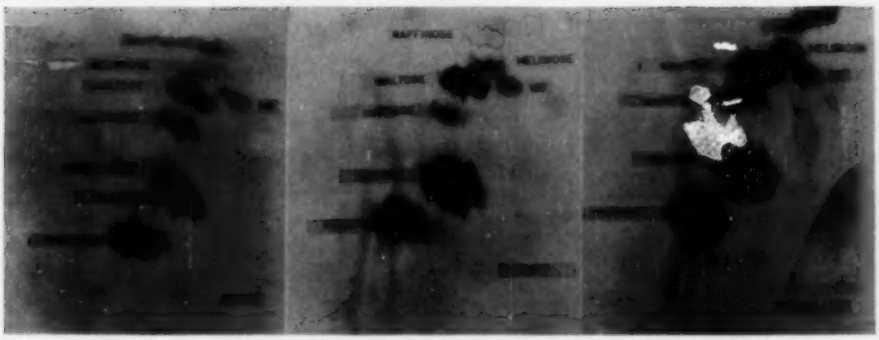

Fig. 4. Two-dimensional paper chromatograms of soluble carbohydrates of intact wheat grains after various germination periods. Amounts used for each chromatogram correspond to 18 mg. of grain (moisture-free basis). Spots are identified with silver nitrate.

unknowns found at any stage of germination. This suggests that the unknown compounds apparently are not normal metabolites, but rather compounds formed during storage after the embryo has lost its viability; these appear to be concerned with the browning reaction characteristic of "sick" wheat.

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THE CARBOHYDRATES OF THE GRAMINEAE

X. A Quantitative Study of the Carbohydrates of Wheat Germ[1]

MICHEL DUBOIS, W. F. GEDDES, AND F. SMITH

ABSTRACT

The embryonic plant and scutellum, obtained by hand-dissection of a soft white wheat, variety Holland, comprised 1.25 and 1.39% respectively of the dry weight of the kernel. The total sugar content of the embryonic plant was 21.9% and of the scutellum 18.4% on a defatted dry matter basis, or 20.1% of the total embryo. Paper chromatography revealed that the sugars consisted almost entirely of sucrose (embryo, 54.7%; scutellum, 62.0%) and raffinose (embryo, 45.3%; scutellum 38.0%) with only traces of glucose. The sugar content of the scutellum decreased when the kernels were exposed to moisture for 1 and 2 days, indicating that the sugar content of the germ may be a useful index of the storage history of wheat.

Commercial wheat germ milled from hard wheats which had been stored several months at 5°C. and then defatted, contained 16.8% total sugars (dry matter basis) consisting of 57.6% sucrose, 37.6% raffinose, 4.8% fructose, and small quantities of glucose.

A single wheat germ may be analyzed by the micro techniques which have been devised.

In 1886 it was shown (22) that wheat germ contained 15 to 18% of soluble carbohydrate material of which the major component was sucrose. A second unidentified, nonfermentable component, which had a high positive specific rotation, was thought to be raffinose, a trisaccharide isolated and crystallized in the same year from barley flour (18).

Several investigations on the composition of wheat germ followed thereafter (9,23,24), and in 1895 commercial wheat germ was shown (25) to contain sucrose and raffinose, both of which were obtained crystalline. In addition a small proportion of glucose was also reported (24) to be present in commercial wheat germ, since the extract exhibited a slight reducing action on Fehling's solution. A quantitative analysis of the carbohydrates in what appears to be the embryonic plant component of the wheat germ was carried out by determining the total extractable carbohydrates (raffinose plus sucrose) and subtracting from this value (24.3%) the percentage of raffinose (6.9%) (deduced from a galactaric (mucic) acid analysis) to give the sucrose content (17.4%).

[1] Manuscript received December 23, 1959. Contribution from the Department of Agricultural Biochemistry, University of Minnesota, St. Paul, Minnesota. Paper No. 4303, Scientific Journal Series, Minnesota Agricultural Experiment Station. The data in this paper form part of a thesis submitted by M. Dubois to the University of Minnesota in partial fulfillment of the requirements for the degree of M.S., January, 1951.

The presence of sucrose and raffinose in commercial wheat germ was placed beyond doubt in 1913 when both sucrose and raffinose were isolated and characterized by melting point and specific rotation (21). The same investigators reported that the embryonic plant component of wheat germ contained a relatively large proportion of glucose, since the aqueous extract of the germ readily yielded glucose phenylosazone. Bearing in mind that phenlyosazone formation is generally performed in an acidic medium, this assertion is open to question since both sucrose and raffinose would readily give rise to glucose phenylosazone.

Crystalline sucrose and raffinose were later isolated from wheat germ and characterized, and it was also reported (4,5) that the raffinose was present exclusively in the embryonic plant. The total carbohydrate content of the wheat germ was 9.4%, a lower value than that hitherto reported; it was said to consist of sucrose 5.2%, raffinose 4.0%, and reducing sugars 0.2%.

As part of a program initiated in these laboratories to study the carbohydrates of the Gramineae, it seemed desirable to reinvestigate the carbohydrates of wheat germ and to resolve, if possible, the difference between the results of Schulze and Frankfurt (25) and those of Colin and Belval (4,5) set forth above.

This paper, briefly reviewed elsewhere (15), is concerned with a study of the carbohydrates of commercial wheat germ and of (a) the embryonic plant (coleoptile-plumule-hypocotyl-coleorhize) portion and (b) the scutellum (scutellum and columnar epithelium) portion of hand-dissected wheat germ, using the modern techniques of column and partition chromatography. Some preliminary experiments have also been conducted on the effect of moisture on the carbohydrates of the wheat germ in the intact kernel.

Materials and Methods

Commercial Germ. Wheat germ milled from hard red spring wheat supplied by General Mills, Inc., Minneapolis, and containing approximately 7.4% moisture was stored in a sealed container at about 5°C. until the various analyses were made. It was relatively free from scutellum material and consisted largely of the embryonic plant contaminated with some flour and appreciable amounts of bran. Before analysis, the germ was ground in a Wiley laboratory mill to pass sieve openings 0.5 mm. in diameter. When the grinding was stopped after only about two-thirds of the material had passed through the sieve, the contamination with bran flecks was greatly reduced.

Hand-Dissected Germ. Since large-scale isolation of pure wheat

germ is impossible, recourse was had to hand-dissection. In this way data were also secured on the percentage of germ present in the kernel. A sample of sound, plump, soft white wheat, variety Holland, containing 9.2% moisture (determined by drying for 1 hour at 130°C.), was employed. Subsamples of the same wheat containing 12.3 and 12.9% moisture, obtained by exposing them in a desiccator containing water at room temperature (24° – 25°C.) for about 18 and 24 hours respectively were also dissected with specially made micro tools. With the assistance of a binocular microscope (30×), the embryonic plant could be separated from the germ by first removing the pericarp covering the germ and applying a slight pressure with a scalpel at point B (Fig. 1). When the kernels contained about 9% moisture, it was a relatively simple matter to obtain the embryonic plant free from the scutellum, but at the higher moistures, it was often contaminated with scutellum. On the other hand, it was difficult to dissect pure scutellum from the wheat kernels at 9% moisture, since the scutellum adhered by its columnar epithelial layer to the endosperm. However, the scutellum was detached from dry kernels by passing the very thin blade of a specially-made scalpel between the scutellum and the endosperm; any endosperm remaining attached to its convex side was removed by careful scraping under the microscope. It was quite difficult to remove all the bran particles from the edge of the shield of the scutellum. The weights of the original kernels, and of the embryonic plant and scutellum, were recorded.

Extraction of Carbohydrates. The commercial wheat germ was dried *in vacuo* at 75°C. over phosphorus pentoxide to constant weight and the loss in weight computed as moisture. The dried sample was

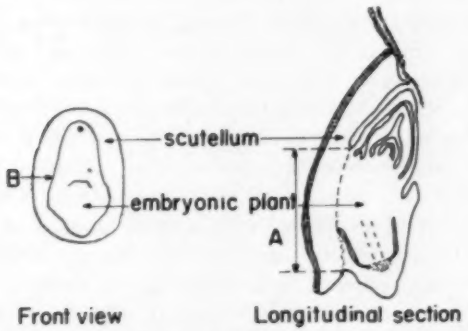

Fig. 1. Diagram illustrating the embryonic plant, A, and the scutellum parts of wheat germ. To remove the embryonic plant, pressure was exerted at B. The scutellum was detached by inserting a specially made scalpel between the scutellum and the endosperm.

extracted with anhydrous diethyl ether in a Soxhlet apparatus for 6 to 7 hours and the percentage of ether-extractable material determined.

(a) Large-scale extraction. For the large-scale isolation of the carbohydrates, 9.665 g. of germ were employed. In order to render the carbohydrate material more accessible to extraction with water, the defatted material was boiled with 95% aqueous ethanol for 1 hour and, without separation, the mixture was freed from solvent by evaporation *in vacuo*. To the residue, 150 ml. of water were added and the mixture boiled for 1.5 hours. The mixture was cooled and centrifuged and the clear yellow liquid evaporated (bath temperature 50° to 60°C.) *in vacuo* almost to a syrup. Absolute ethanol was added to precipitate protein, which was removed by centrifugation. The clear solution was reconcentrated as before and the treatment with ethanol repeated. The process of concentration and treatment with ethanol was repeated until no more precipitate was produced. Each precipitate was redissolved in water and examined for the presence of sugars by paper chromatography. If any were detected, the solution was treated with ethanol to precipitate impurities; the sugars in the mother liquor were recovered by evaporation and combined with the main bulk of the material. During all the manipulations the pH was checked and the solutions maintained neutral or slightly alkaline by the addition of a little dilute ammonium hydroxide to prevent any hydrolysis of the sucrose and raffinose during the concentrations.

Evaporation of the aqueous ethanol solution *in vacuo* (bath temperature 50° to 60°C.) gave the soluble carbohydrates as a white amorphous powder which was dried *in vacuo* to constant weight.

(b) Micro-extraction. For the micro-extractions, about 100 mg. of commercial wheat germ, 15 to 20 mg. of hand-dissected embryonic plant material, and 10 to 15 mg. of the scutellum were employed. After drying *in* vacuo at 75°C. over phosphorus pentoxide and removal of most of the lipid by extraction with absolute diethyl ether, the residue was boiled with 95% aqueous ethanol for 1 hour and the solvent evaporated without separation. The extraction of the sugars was carried out with about 1 ml. of boiling water. The extract was filtered and the residues washed with a small amount of water. Losses were largely eliminated by carrying out the successive extractions with diethyl ether, ethanol, and water in the same vessel (Fig. 2). The combined extract and washings were passed first through a weak cation exchange resin (Duolite C3) and then through an anion resin (Duolite A4). The eluate was concentrated *in vacuo* to dryness and weighed.

To determine whether the resins used for the removal of protein and inorganic material absorbed any of the sugars from the aqueous extract of the wheat germ, the following experiments were conducted:

(a) Aqueous solutions containing known amounts of D-glucose, sucrose, and raffinose were passed successively through the resins and, after adjustment of the solution to a known volume, the sugar in the final eluate was determined by the phenol sulfuric acid method (see below). Repeated experiments showed that recovery was quantitative.

(b) An aqueous solution containing a mixture of 500 γ of sucrose and 500 γ of raffinose was added to a 10-ml. aliquot from 25 ml. of aqueous solution containing the carbohydrates from 0.0228 g. of embryonic plant. The 10 ml. of aqueous extract of the germ with and without the added sugars were treated with the two resins and the solutions analyzed after concentration for sucrose and raffinose by paper chromatography using the phenol-sulfuric acid reagent as described in the next section. The difference between the two results indicated that the recovery of sucrose and raffinose was 99%.

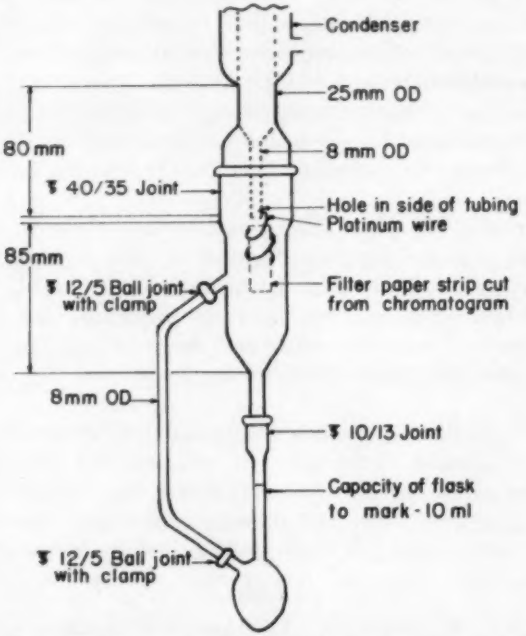

Fig. 2. Diagram of apparatus employed for the microextraction of lipids and sugars from wheat germ tissues.

Paper Chromatography. (a) Qualitative. Whatman No. 1 filter paper was used throughout, some papers being freed from soluble carbohydrate impurities by washing (13). The following solvents were employed: A, phenol saturated with water (irrigation time, 15 to 18 hours) (19); B, 1-butanol:propionic acid:water (4:1:5) (irrigation time 70 hours) (3); C, 1-butanol:ethanol:water (4:1:5) (irrigation time 70 hours) (19). The sugars were detected on the chromatograms by spraying either with ammoniacal silver nitrate and heating for 1 to 2 minutes at 100°C. (19) or with a solution of N,N′-dimethyl-*p*-aminoaniline (0.2 g.) in 50% aqueous ethanol (50 ml.) containing trichloroacetic acid (1 g.) and conc. hydrochloric acid (1 ml.) and heating for 5 to 10 minutes at 130°C. (3).

(b) Quantitative. The mixture of sugars was separated by solvent A and the components were determined by the phenol-sulfuric acid reagent (7,8), reference being made in the usual way to standard curves for fructose, sucrose, and raffinose (7). The absorbance of the colored solution formed by the reaction of the sugars with the phenol-sulfuric acid reagent was determined in a Beckman DU spectrophotometer at 490 $m\mu$.

In one experiment, the sugars from commercial wheat germ were separated by paper chromatography and determined by means of Dreywood's anthrone reagent (6,16).

Microanalysis of Sugars Separated by Paper Chromatography. This procedure was adopted for the separation of as little as 200 γ of sugars in order to permit the analysis of the sugars in a single, hand-dissected wheat germ.

The section of the paper chromatogram containing the sugar component to be analyzed was excised, rolled up, and suspended from the end of a reflux condenser with a platinum loop (see Fig. 2). Water (5 ml.) was placed in the flask and the apparatus assembled. The water was boiled under the reflux and drops of liquid fell from the condenser onto the paper held in the loops and the sugars were extracted.[2]

After 30 minutes of refluxing the extract was allowed to cool and the volume adjusted to 10 ml. The solution was filtered through sintered glass and a suitable aliquot (2 ml.) of this solution was treated with 80% phenol (0.1 ml.) and sulfuric acid (5 ml.), and the absorbance of the colored solution determined as already described (8).

Results

Commercial Wheat Germ. The commercial wheat germ which

[2] The present technique being followed in these laboratories for extracting the sugars from paper chromatograms is described in the paper by Dubois *et al.* (7).

had been stored at 7.3% moisture (determined by drying *in vacuo* at 75°C. over phosphorus pentoxide) for several months at about 5°C. contained 14.9% lipid (dry matter basis, determined by extraction with diethyl ether).

Chromatograms irrigated with the three solvent systems employed and developed with ammoniacal silver nitrate or with the N,N'-dimethyl-*p*-aminoaniline reagent revealed the presence of sucrose and raffinose (major components) and glucose and fructose (minor components). The quantitative data obtained by the various methods are summarized in Table I.

The mean quantity of total sugars extracted was 16.8% and the results (apart from one fructose analysis) obtained by the micro and semimicro extraction techniques are in good agreement. The mean composition of the sugars was sucrose 57.6%, raffinose 37.6%, and fructose 4.8%. However, some glucose was present in all samples of sucrose which were separated by paper chromatography using Solvent A (Table I). These results confirm reports in the literature that the major carbohydrate components of commercial wheat germ are sucrose and raffinose but small quantities of reducing sugar are present (24,25).

Isolation of Crystalline Sucrose. Evaporation of the aqueous solution of the component of commercial wheat germ corresponding to sucrose which was obtained by chromatography on a cellulose column

TABLE I

THE CARBOHYDRATES OF COMMERCIAL WHEAT GERM

					MEAN
Weight of germ extracted (dry matter basis) g.		9.665		0.100	
Total sugars extracted, % d.m. basis		16.9		16.7	16.8
Component sugar, %					
Fructose	5.6	6.2	4.7	2.7	4.8
Sucrose	56.6	55.3[a]	57.4	61.1	37.6
Raffinose	37.8	38.5	38.0	36.2	57.6
Chromatographic analysis					
Method	Column (2)[b]	Paper (7)	Paper (7)	Paper (7)	
Solvent[c]	A	B	A	A	
Sugar reagent	Direct weighing	Anthrone-sulfuric acid (6)	Phenol-sulfuric acid (7)	Phenol-sulfuric acid (7)	

[a] Paper chromatographic analysis using solvent B showed that some glucose was present in all samples of sucrose separated. In this case 7.5% of the total sugars consisted of glucose.

[b] Numerals in parentheses refer to references in "Literature Cited."

[c] Solvent A = phenol saturated with water, B = 1-butanol:ethanol:water (4:1:5).

(2,12) gave a syrupy product which crystallized spontaneously. The residue was dissolved in the minimum amount of water and ethanol was added until a faint turbidity was produced. After 1 day in an open container, slow evaporation gave crystalline sucrose. After the mother liquor was removed, crystallization from aqueous ethanol gave pure sucrose m.p. and mixed m.p. 184°C. $[\alpha]_D^{22}$ +64.4° in water (c, 1.0).

Isolation of Crystalline Raffinose. The fraction of the carbohydrate material extracted from commercial wheat germ whose R_F value corresponded to raffinose crystallized spontaneously. Crystallization first from aqueous ethanol as described above for sucrose and then from water yield pure raffinose m.p. and mixed m.p. 125°C., $[\alpha]_D^{23}$ +99.5° in water (c, 0.5).

Hand-Dissected Wheat Germ. The results obtained by dissection and analysis of soft white wheat, variety Holland, are recorded in Table II. The total embryo comprised 2.64% by weight of the kernel (dry matter basis). The percentage of germ or embryo reported in the literature varies rather widely. Thus, Girard (10) reported values from 1.16 to 1.50% for four French wheats; Osborne and Mendel (17) in 1919 reported 1.5%, presumably for American wheat; Percival (20) recorded 2.8 to 3.5%, and Grischenko (11) found values ranging between 2.56 and 3.25% for six samples representing two varieties of Russian wheat. Bailey (1) determined the germ content of several samples of hard red spring, hard red winter, durum, and soft wheats grown in Canada or the United States. The average germ content for the soft wheats was 2.66%, and the average for all wheats was 2.61%. The germ content obtained in the present study is in good agreement with these values. The embryo component comprised 47.5% and the scutellum 52.5% of the total weight of the germ.

Sucrose and raffinose were the only sugars detected by paper chromatography when the chromatograms of the extracts were sprayed with N,N′-dimethyl-*p*-aminoaniline trichloroacetate; when sprayed with ammoniacal silver nitrate, trace amounts of glucose were detected in addition to sucrose and raffinose. No fructose could be detected even when the carbohydrate mixture was put on the chromatogram as a syrup.

The analyses for total sugars in Table II agree quite well in view of the fact that only 10 to 23 mg. of material were extracted. The low value for the total sugars in the embryonic plant obtained in experiment 1 was probably due to losses during manipulation. In subsequent experiments the losses were largely eliminated by carrying out the successive extractions with diethyl ether, ethanol, and water in the same vessel.

The sugar content of the total embryo of 20.1%, expressed on a defatted moisture-free basis for the hand-dissected material, agrees much more closely with the value of 24.3% reported for wheat germ by Schulze and Frankfurt (25) than the value, 9.2%, recorded by Colin and Belval (4,5); however, the proportions of sucrose, 56.5%, and raffinose (43.5%) deduced from their analyses are in quite good agreement with those in Table II. Schulze and Frankfurt (25) found that raffinose comprised 28.3% of the sugars of the germ.

Effect of Varying the Moisture Content of Wheat on the Sugars in the Germ. The analyses in Table III show that when wheat kernels are moistened the content of total sugars in the embryonic plant and particularly in the scutellum decrease, probably because of translocation or increased respiration. The decrease is very striking since

TABLE II

CARBOHYDRATES OF THE EMBRYONIC PLANT AND SCUTELLUM FRACTIONS OBTAINED BY HAND-DISSECTION[a]

	Proportion of Kernel	Lipid Content[b]	Total Sugars[a]	Component Sugars (Percent of Total)	
				Sucrose	Raffinose
	%	%	%	%	%
Embryonic plant[c]					
1			19.3	54.0	46.0
2			22.1	52.8	47.2
3	1.25	15.3	23.3	55.7	44.3
4			23.0	56.4	43.6
Mean			21.9	54.7	45.3
Scutellum					
1	1.39	12.6	19.1	62.2	37.8
2			17.6	61.7	38.3
Mean			18.4	62.0	38.0
Total embryo[d]	2.64		20.1	58.5	41.5

[a] Expressed as percent of defatted germ, dry matter basis. Replicate No. 4 was carried out with only 1.44 mg. of sample.
[b] Diethyl ether.
[c] The average weight of the embryonic plant component of a single wheat kernel was 0.48 mg.
[d] Calculated.

TABLE III

EFFECT OF VARYING THE MOISTURE CONTENT OF WHEAT ON THE SUGARS IN WHEAT GERM[a]

	Moisture, 9.2%		Moisture, 12.3%		Moisture, 12.9%
	Scutellum	Embryonic Plant	Scutellum	Embryonic Plant	Scutellum
Total sugars, %	18.4	22.0	11.8	17.9	13.6
Sucrose, % of total	62.0	54.7	57	56	51
Raffinose, % of total	38.0	45.3	43	44	49

[a] Sugar content is expressed on a dry-matter, defatted-tissue basis.

the kernels at 12.3 and 12.9% moisture were only stored over water for 18 and 24 hours respectively. These limited observations indicate that the ratio of sucrose to raffinose remains constant in the embryo but decreases in the scutellum upon short-time storage of the wheat at 12.3 to 12.9% moisture content.

Discussion

Commercial wheat germ is contaminated with some bran and flour and the lower total sugar content (16.8%, d.m. basis) of the sample used in these studies in comparison with that (20.1% d.m. basis) of the total embryo of hand-dissected germ would be expected. The sugars of hand-dissected germ consisted almost entirely of sucrose (58.5%) and raffinose (41.5%), although trace amounts of glucose were also detected when the chromatograms were sprayed with ammoniacal silver nitrate. Although the sugars of the commercial wheat germ also consisted mainly of sucrose (57.6%) and raffinose (37.6%), fructose (4.8%) along with some glucose was present. It thus appears that the carbohydrates of sound wheat germ are sucrose and raffinose and that the glucose and fructose in commercial wheat germ probably result from the partial hydrolysis of sucrose and raffinose. It is significant that upon storage of the wheat over water for 18 to 24 hours the sucrose content of the scutellum decreased. Recently, Linko *et al.* (14) have reported that fresh granular wheat germ milled in the United States contained 28.6% total sugars (dry matter basis) consisting principally of sucrose (55.9%), raffinose (38.1%), fructose (2.8%), glucose (2.1%), and meliboise (1.1%). The total sugar content of this sample was not expressed on a defatted basis and the results are therefore appreciably higher than those obtained in the present study. The relative percentages of sucrose and raffinose are, however, in close agreement. It appears that the carbohydrates of sound pure wheat germ are sucrose and raffinose and the glucose, fructose, and melibiose reported in commercial wheat germ probably result from the partial hydrolysis of sucrose and raffinose. This observation is in agreement with the results of many studies in these and other laboratories which have shown that storage of wheat at moisture contents above about 14.5% (wet basis) results in a progressive decrease in nonreducing sugar content. It is possible that the sugar content and nature of the sugars in wheat germ may be used as an index of the storage history of wheat.

The above investigations have also revealed that micro amounts of wheat germ in the order of 10 to 20 mg. can be analyzed with satisfactory accuracy and micro extraction and analytical procedures

may be employed to study the sugar contents of germs from individual kernels.

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GRAIN STORAGE STUDIES

XXXI. Changes Occurring in Low-Molecular-Weight Compounds in Deteriorating Wheat[1]

R. L. GLASS AND W. F. GEDDES

ABSTRACT

D-galactose, myo-inositol, and glycerol were isolated and characterized from the monosaccharide fraction obtained from a sample of wheat which had deteriorated under an atmosphere of nitrogen for 24 weeks at 18% moisture and 30°C. In addition, D-glucose and D-fructose were obtained in chromatographically pure form. The control wheat sample yielded D-glucose and D-fructose together with trace amounts of what appeared to be D-galactose and myo-inositol.

In a recent paper in this series (5), marked changes in reducing and nonreducing sugars were reported in wheat stored at 16–18% moisture in an atmosphere of nitrogen. Nonreducing sugars (expressed as sucrose) decreased greatly and to an extent which was almost exactly compensated for by an increase in reducing sugars (expressed as maltose). When damp wheat was stored in air, and hence became exceedingly moldy, the increase in reducing sugars was only about one-fourth as great as the decrease in nonreducing sugars. Apparently sugars were extensively utilized by molds.

Expressing the nonreducing and reducing sugars as sucrose and maltose, respectively, is purely arbitrary and no indication of the actual sugars involved is implied.

Sucrose, along with raffinose, is a prominent constituent of wheat

[1] Manuscript received December 31, 1959. Presented at the 43rd annual meeting, Cincinnati, Ohio, May 1958. Contribution from the Department of Agricultural Biochemistry, University of Minnesota, St. Paul, Minnesota. Paper No. 4307, Scientific Journal Series, Minnesota Agricultural Experiment Station.

germ (3) but is only a minor component (0.1 to 0.2%) of wheat flour (8,10). Wheat flour contains about 1% of glucofructans which are readily hydrolyzed with dilute acids and hence contribute markedly to the nonreducing sugar values, as determined by conventional methods using acid hydrolysis (1). Although the reducing sugars in wheat are usually reported as maltose for convenience, it is well known that glucose and fructose are also present (6,8,10).

The objective of the present study was to determine the nature of the reducing sugars which are formed when moist wheat is stored in an atmosphere of nitrogen. In the course of this investigation, the substances present in a monosaccharide fraction obtained from a sound and a deteriorated sample of wheat by elution from charcoal were studied.

Materials and Methods

Two samples from the same original lot of Marquis wheat were used. One had been stored for 24 weeks at 30°C. and 18% moisture under an atmosphere of nitrogen (containing 0.04% oxygen); the other was a control sample which was stored at −10°C. during the same period. The viability, nonreducing and reducing sugar contents were:

	Viability	*Sugars per 10 g. wheat (dry basis)*	
		Nonreducing (as sucrose)	*Reducing (as maltose)*
	%	*mg*	*mg*
Control	97	232	37
Stored under nitrogen	0	75	120

Extraction of Sugars: Each sample, air-dried and ground to pass a 30-mesh screen, was defatted by extraction with petroleum ether. Eleven hundred grams of the defatted material were suspended in boiling 70% (v/v) aqueous ethanol and refluxed for 2 hours to inactivate the enzymes. The centrifuged material was extracted three times with successive 3-liter portions of warm (70°C.) 70% ethanol. All extracts were pooled and evaporated under reduced pressure at 40°C. to a thick syrup. The syrup was dissolved in 600 ml. distilled water and dialyzed against 2 liters of distilled water at approximately 5°C. for 72 hours. The dialysate was then decreased in volume to 100 ml. under reduced pressure at 40°C. and deionized by passage through an Amberlite MB-3 resin[2] column.

[2] Fisher Scientific Co., Pittsburgh, Pa.

Preparation of the Monosaccharide Fraction with Charcoal (11). One hundred grams each of charcoal (Darco G60)[3] and Celite 535[4] were suspended in 2.5% aqueous ethanol[5] and the slurry poured into a chromatographic column to give, when settled, a column of adsorbant 35 mm. × 350 mm. A 25-ml. aliquot of the sugar concentrate (adjusted to a concentration of 2.5% ethanol by the addition of absolute ethanol) was added to the column and developed by the addition of 2 liters of 2.5% aqueous ethanol, after which an essentially negative Molisch test was obtained on the eluate. The material was then evaporated to a syrup and stored at −10°C. until used.

Paper Chromatography. Paper chromatograms of the material eluted from the charcoal by 2.5% ethanol, along with known sugars, were obtained by spotting on strips of Whatman No. 1 filter paper and developing in the descending manner for periods ranging from 24 to 48 hours, using the solvent system 1-butanol-pyridine-water (6:4:3) (v/v). The positions of the sugars were located by spraying the air-dried chromatograms with ammoniacal silver nitrate and heating in the usual manner.

Column Chromatography. The compounds present in the fraction obtained from the nitrogen-stored wheat were isolated by chromatography using a column of cellulose powder (Whatman No. 1). Approximately 400 mg. of the syrup were dissolved in a minimum of 1-butanol-pyridine-water (6:4:3) (v/v) and applied to the column (35 mm. × 600 mm.); development of the column was carried out using this solvent system. Fractions (4 ml. per tube) were collected by means of an automatic fraction collector. Those tubes which contained material reacting with ammoniacal silver nitrate were detected by spotting a drop from each tube on a piece of filter paper, spraying with the reagent, and heating. The material in the tubes containing identical compounds, as determined by paper chromatography, were pooled and the solvent was removed by evaporation under reduced pressure at 40°C.

Results and Discussion

The material obtained by extraction of sound and spoiled wheat and partial fractionation on charcoal gave, when subjected to paper chromatography, the following results.

[3] Atlas Powder Co., Wilmington, Del.

[4] Johns-Manville Co., New York, N. Y.

[5] Dilute aqueous ethanol (2.5%) rather than water was used, since this gave a better separation of mono- and disaccharides.

Sample	*Component Number*						
	1	2	3	4	5	6	7
	Rg value[a]						
Control	...[b]	0.61	0.77	...[b]	1.0	1.16	...
Stored under nitrogen	0.26	0.61	0.77	0.85	1.0	1.16	1.75

[a] Rg represents the mobility of the compound in question with relative respect to that of glucose.
[b] Present but in barely detectable amounts.

Of the seven substances detected in more than trace amounts in the deteriorated wheat, only four appeared in the control sample. Comparison with known sugars indicated components 2, 3, 5, and 6 to be maltose, sucrose, glucose, and fructose respectively, sugars which have been shown to be present in wheat flour (6,8,10). Components 1, 4, and 7 from the sample stored under nitrogen were obtained in chromatographically pure form by separation on the cellulose column and were identified as follows:

Component 1. Crystallized from methanol. Identified as myo-inositol by m.p. 225°C., mixed m.p. with myo-inositol 225°C. Preparation of hexaacetate by treatment with acetic anhydride in anhydrous pyridine. Melting point and mixed melting point 219°–220°C.

Component 4. Crystallized from methanol. Identified as galactose by m.p. and mixed m.p. with authentic sample 162°–164°C.; N-methylphenylosazone prepared by treatment with N-methylphenylhydrazine, m.p. and mixed m.p. 192°C.

Component 7. A viscous, slightly brown liquid which gave an odor of acrolein when heated with sodium bisulfate. Identified as glycerol by preparation of glyceryl tri-*p*-nitrobenzoate by treatment with *p*-nitrobenzoyl chloride in pyridine; m.p. and mixed m.p. 195°C.

D-galactose, myo-inositol, and glycerol obtained from the deteriorated wheat have not, to the authors' knowledge, been reported previously to be present in the free state in sound wheat. Subsequent to the completion of this work, Linko *et al.* (7), in storage experiments with wheat germ and intact wheat, observed characteristic increases in reducing sugars, principally fructose, glucose, and galactose, and a decrease in nonreducing sugars, primarily raffinose. They also observed a transient appearance of several other unidentified sugarlike compounds. Galactose has also been reported to be present in autolytic extracts of barley (9). Chromatographic evidence in the present study indicated that D-galactose and myo-inositol are present in sound wheat, but in such small amounts that no attempt was made to isolate them for more positive identification.

Some wheat enzymes are quite active at moisture contents of 18% and 30°C. (5), and wheat contains phytase and lipase. The hydrolysis

of phytic acid would result in free myo-inositol and an increase in inorganic phosphate. The latter has been reported in a previous paper (4). The presence of free glycerol indicates that wheat lipase can catalyze the hydrolysis of triglycerides in both the alpha- and beta-position of the glycerol moiety.

The source of free galactose in this wheat is less certain. Several galactose-containing compounds are known to be present in wheat. These include raffinose (3), and galactosyl glycerides (2). The galactose found here could conceivably have originated from either of these compounds. A demonstration of the presence of enzymes in wheat capable of hydrolyzing these compounds would help clarify this point.

Glucose, fructose, sucrose, and maltose have been isolated and identified from wheat flour by other workers (6,8,10) and were not further identified here.

A quantitative study of the changes in individual sugar levels in deteriorated wheat is currently underway. Visual comparison of chromatograms of extracts of sound and deteriorated wheat in the present study indicate that little or no change has occurred in the maltose and sucrose levels.

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DISAPPEARANCE OF BROMATE DURING BAKING OF BREAD[1]

W. Bushuk and I. Hlynka
With addendum by
C. C. Lee[2] and R. Tkachuk

ABSTRACT

The amperometric titration method was applied to the study of the disappearance of bromate during baking of bread from bromated flour. For initial potassium bromate concentrations of 5 to 40 p.p.m., essentially no bromate was found in the bread crumb after a 10-minute baking period. For 80 p.p.m. a small amount of bromate was found after 15 minutes baking time; none was found after 20 and 25 minutes. Similar results were obtained for bread baked according to the formula of Lee *et al.* from flour containing 20 and 40 p.p.m. potassium bromate. Additional experiments by Lee and Tkachuk have reconciled their previous findings with those of this study.

Until recently, it has been the opinion of most cereal chemists that there was no residual bromate in bread baked from flour containing normal amounts of potassium bromate (4,5,8). In a recent study, Lee and Tkachuk (6) found that bread baked from flour treated with 5 to 30 p.p.m. of Br^{82}-labeled potassium bromate contained residual Br^{82} equivalent to 33 to 42% of the bromate added. They concluded that the residual radioactivity was due to bromate.

The present study was designed to reinvestigate the decomposition of bromate during the baking procedure and to check the presence of bromate in bread by the amperometric titration technique (2,3). The results that were obtained with initial concentrations from 5 to 80 p.p.m. of potassium bromate are reported in this paper.

Materials and Methods

The flour used in this study was a straight-grade flour, milled commercially from hard red spring wheat. It was described completely in a previous paper (2). The following baking formula was employed: 100 g. flour (14% moisture), 2.0 g. fresh yeast, 2.5 g. sucrose, 1.0 g. salt, 0.1 g. ammonium dihydrogen phosphate, 0.3 g. nondiastatic malt (250° Lintner), potassium bromate as required, and water to give 59.5% absorption. A separate dough was prepared for each bromate determination. The doughs were mixed, fermented, and proofed according to the basic baking test procedure (1). For comparison purposes, experiments with 0, 20, and 40 p.p.m. (flour basis) of potassium

[1] Manuscript received February 3, 1960. Paper No. 183 of the Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg 2, Canada.
[2] Department of Chemistry, University of Saskatchewan, Saskatoon, Sask., Canada.

bromate were made using the baking formula of Lee, Tkachuk, and Finlayson (7), which includes shortening and milk solids in addition to the materials listed above. The oven temperature was 220°C. in all baking experiments.

Ten-gram subsamples of dough (or bread crumb) were analyzed for bromate by the amperometric titration method (2,3) immediately after each of the following stages: mixing, first punch, second punch, panning and proofing, and at 5-minute intervals during baking. The quantity of dough (or crumb) was reduced to 10 g. to increase the clarity of the extracts, particularly in baked bread. Suspended materials in the extracts do not affect the end point, but tend to decrease the slope of the titration curve (μamp. vs. ml. of titrant) and accordingly decrease the accuracy of the titration. Three bromate analyses were made using the extraction procedure of Lee and Tkachuk (6) except that the treatment with bromine and extraction with carbon tetrachloride was omitted. All results were reported in parts of potassium bromate per million of flour containing 14% moisture.

Results and Discussion

Table I gives the amounts of bromate found in dough, given in p.p.m. of flour immediately after the steps in the baking procedure indicated. The amounts recovered after the loaves were put in the oven were not corrected for the drop in moisture during baking; the actual correction that could be applied is negligible, since the drop in moisture content of crumb during baking was only about 1%.

TABLE I

BROMATE RECOVERIES FROM DOUGH AND BREAD

Baking Stage[b]	Bromate Added (p.p.m. flour)						
	5	10	20	20[a]	40	40[a]	80
	Bromate Recovered (p.p.m. flour)						
Mix	4.61	9.73	19.46	17.92	36.10	34.30	73.73
First punch	4.10	8.70	17.15	16.90	33.28	32.00	68.10
Second punch	3.97	8.45	16.90	16.64	32.26	32.26	67.07
Pan	3.97	8.20	16.64	16.13	32.00	31.49	66.05
Proof	3.58	7.94	16.38	15.36	31.23	29.70	64.26
Oven (minutes)							
5	3.97	3.84	11.01	4.99	21.50	23.55	51.71
10	0.77	0.0	0.0	1.28	0.0	11.26	18.94
15	0.0	0.0	0.0	0.0	0.0	1.54	2.56
20	0.0	0.0	0.0	0.0	0.0	0.0	0.0
25	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a] Baking formula of Lee *et al.* (7).
[b] Analyses were made immediately after the various stages indicated.

The results of Table I show unequivocally that for initial potassium bromate concentrations up to 80 p.p.m., no bromate is demonstrable in bread after a 25-minute baking period. For 40 p.p.m. potassium bromate or lower, no bromate could be detected in the bread 10 minutes after the dough entered the oven. Essentially the same results were obtained for loaves prepared according to the formula of Lee and Tkachuk (6) containing initially 20 and 40 p.p.m. of potassium bromate. There is an indication that the length of time necessary for all the bromate to react increases with increasing initial bromate concentration; accordingly, it is quite possible that at some concentration higher than 80 p.p.m. part of the bromate would remain in the bread. For example, Freilich and Frey (4) found small amounts of the bromate in bread for the initial potassium bromate concentration of 100 p.p.m. and larger amounts in loaves containing initially 500 and 1,000 p.p.m. However, these concentrations exceed those encountered in baking practice.

The presence of bromate in the aqueous extract (prior to treatment with bromine and extraction with carbon tetrachloride), prepared according to the procedure of Lee and Tkachuk (6) from bread made from flour containing 30 p.p.m. potassium bromate, was checked by the amperometric titration method. No bromate could be detected. Potassium bromate, added to the bread crumb during extraction in the amount equivalent to 5 p.p.m., was completely recovered and there were no interfering substances extractable from bread that contained initially no bromate.

There seemed to be an obvious discrepancy between the results of this study and those of Lee and Tkachuk (6). A copy of this manuscript was made available to these authors at the time it was submitted for publication. In an attempt to reconcile the two sets of results Lee and Tkachuk undertook to check their results by further experiments with Br^{82}-labeled bromate. Their findings, which bring the previous results in line with those of this study, are included as an addendum.

Addendum by C. C. Lee and R. Tkachuk

A repetition of the work on the presence of bromate in bread baked with Br^{82}-labeled bromate but using a flour different from that employed previously (6) showed that there is no residual bromate in bread. The error in the earlier work (6) was likely due to an incomplete exchange of bromide with bromine under the conditions of those experiments. Although control tests showed that aqueous solutions of bromide exchanged readily with bromine, when bread was baked from dough containing Br^{82}-labeled potassium bromide, the bromide recovered in the aqueous bread extract could not be removed completely by one simple exchange with bromine.

For example, with the aqueous extract from bread originally containing 30 p.p.m. Br^{82}-labeled potassium bromide, one exchange treatment followed by extraction of the bromine with organic solvent removed only about 80% of the bromide. The residual bromide, however, could be removed by a second exchange treatment with bromine. Since no successive exchange treatments were carried out in the previous work (6), the bromide which was not removed by one exchange with bromine was mistakenly assumed to be bromate.

Acknowledgments

The authors wish to thank F. D. Kuzina who did the baking, and R. A. McLeod for the bromate analyses.

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A NOTE ON THE ENZYMATIC REDUCTION OF PROTEIN IN HIGH-AMYLOSE CORN STARCH[1]

Charles Vojnovich, R. A. Anderson, and E. L. Griffin, Jr.

Recent articles published by the Northern Regional Research Laboratory on experimental wet-milling of high-amylose corns containing starch with 49 to 57% amylose indicate that recovery of low-protein starch presents some difficulty (2,3). In the experimental milling where starch-gluten separation was carried out on starch tables, double

[1] Manuscript received January 23, 1960. Contribution from the Northern Regional Research Laboratory, Peoria, Illinois. This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

tablings were necessary to obtain a fair separation. While it would be expected that better results would be obtained by using centrifuges and other machinery commonly found in the starch industry, it seemed of interest to investigate another method of reducing the protein content of this starch. The treatment of corn starch with a proteolytic enzyme to reduce the protein content has been described in the patent literature (4). In this procedure, the proteins in the starch were made soluble by the enzyme pepsin, and were removed by decantation and filtration.

This paper reports results of a short study on the effect of certain variables in the reduction of proteins by the enzymatic action of trypsin and papain on high-amylose corn starch (57% amylose content).

The starting material was prepared from corn with starch containing 57% amylose by the small-scale wet-milling procedure described by Anderson *et al.* (3).[2] The starches used in this work were from the first tabling of the mill starch, and their protein contents varied from 0.81 to 1.27%. In all experiments 50 ml. of starch slurry (18.7 – 19.6% dry solids) were subjected to the enzyme treatments. One-percent stock solutions of trypsin and papain[3] were prepared by dissolving the enzyme in distilled water and filtering it to remove any sediment. The bases of selection for these enzymes were their commercial availability, relatively high purity, and freedom from amylases, etc. Because of the limited scope of the study, other enzymes were not investigated.

The experiments were carried out in the following manner: The pH of the starch slurry was adjusted to the desired level with either 1*N* acetic acid or 1*N* sodium hydroxide, the enzyme solution was then added, and the mixture was held at the specified temperature for the required period of time, with occasional shaking. Continuous agitation had negligible effect on these small samples. The starch slurry was then filtered, washed, and dried. A control sample was included in each test.

The moisture in the starch was determined by drying a sample for 4 hours at 100° – 110°C., under a vacuum of 28 in. of mercury. Protein ($N \times 6.25$) was determined by the improved Kjeldahl method for nitrate-free samples (1).

Like most proteolytic enzymes, trypsin and papain have an opti-

[2] Corn was provided by the American Maize-Products Co., Roby, Indiana, and the National Starch and Chemical Corp., Plainfield, N. J. The corn was grown from strains developed by Bear Hybrid Corn Co., Decatur, Ill.

[3] Difco trypsin (Ref. No. 359773) and purified papain (Meer Standard Lot 8-5050XXP). Use of these enzymes does not imply that they are endorsed or recommended by the U. S. Department of Agriculture over others not mentioned.

mum pH range in which their hydrolytic activity is at its greatest. Experiments conducted to establish the proper pH for carrying out each protein hydrolysis showed that in the pH range 4.3 – 6.1 papain reduced the protein content of the starch from 0.81 to 0.60%. A higher pH range, 6.1 – 8.8, was found suitable for trypsin, with the protein content reduced from 0.81 to 0.52 – 0.56%.

As little as 0.2% of trypsin was found to reduce the protein content of the starch from 1.27 to 0.42%. When the enzyme concentration was increased to as high as 2.0%, no further protein reduction was attained. An addition of 0.5% of papain resulted in a reduction of protein from 1.27 to 0.56%. As noted with trypsin, increasing the enzyme concentration had no effect on protein reduction.

It was noted that both papain and trypsin could be used effectively at a wide range of temperatures to reduce protein content. The greatest enzyme activity occurred with both enzymes at a temperature of 40°C. The best protein reduction was obtained with trypsin, where the protein in the high-amylose starch was reduced from 1.27 to 0.42%.

Proteolytic activity started almost immediately upon addition of the enzymes to the starch slurry and hydrolysis of the protein was completed in about 2 hours. When papain was used, a reduction in protein content from 0.81 to 0.69% was realized in 2 hours of hydrolysis; in 16 hours, 0.66% of protein remained in the starch. Similarly, in 2 hours trypsin reduced the protein content of the starch to 0.52%; the same reduction was attained after 16 hours of proteolysis.

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ERRATA

Cereal Chemistry, Vol. 37, No. 3
(May, 1960)

Page 241, Donelson and Wilson:
Page 247, 2nd equation of set in middle of page; *for* $x_2 + \text{gluten}/ \ldots$ *read* $x_2 = \text{gluten}/ \ldots$
Page 254, Table V, fourth column heading from left; numerator and denominator are reversed and the heading should read:

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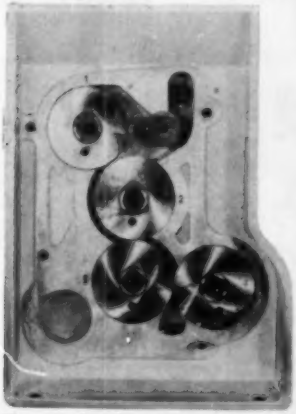

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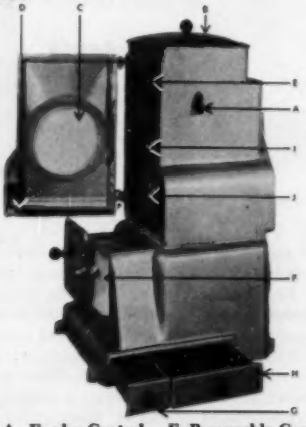

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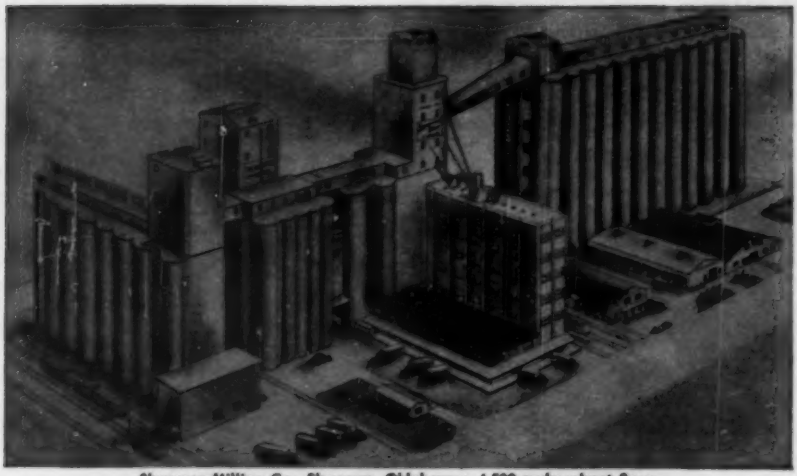

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